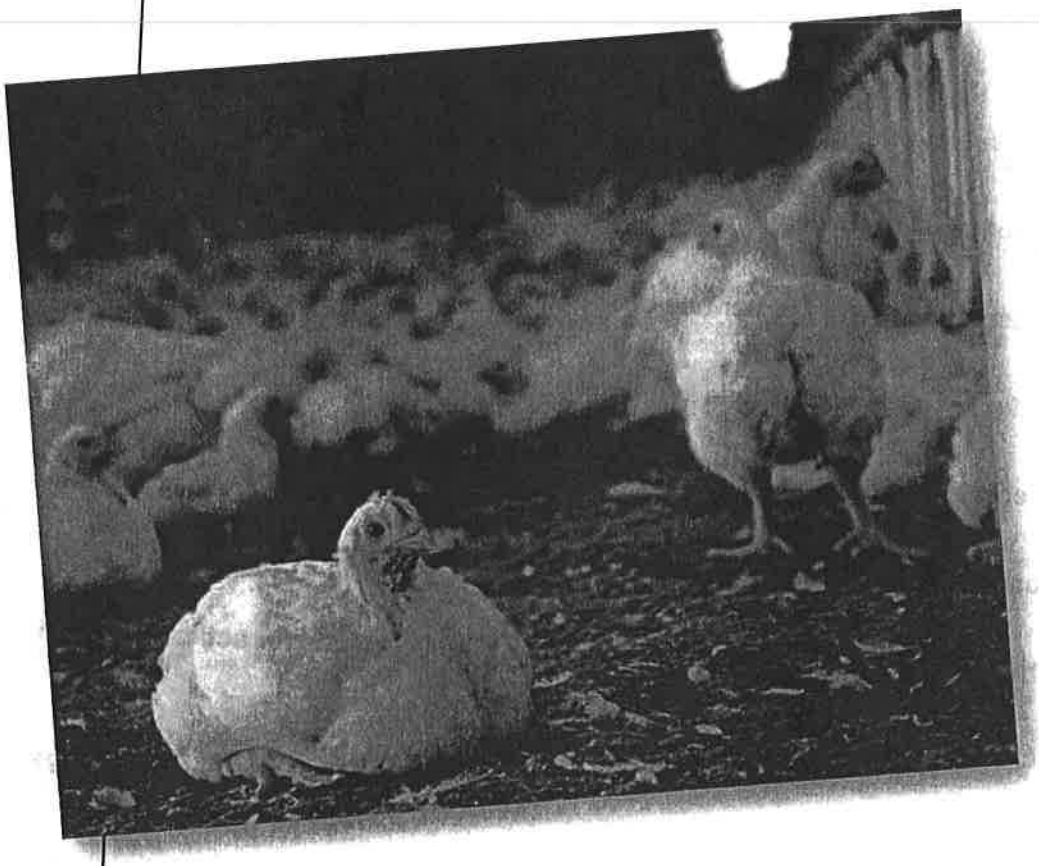


Chapter 4.

Reducing the use of antibiotics in animal husbandry

Contents

Chapter 1 - The evolving threat of antimicrobial resistance - Introduction 1

Chapter 2 - Surveillance to track antimicrobial use and resistance in bacteria 11

Chapter 3 - Measures to ensure better use of antibiotics 31

Chapter 4 - Reducing antimicrobial use in animal husbandry 49

Chapter 5 - Infection prevention and control in health-care facilities 63

Chapter 6 - Fostering innovation to combat antimicrobial resistance 77

Chapter 7 - The way forward: political commitment to enable options for action 91

References 95

Appendices 109

Appendix 1: List of 2001 WHO Global Strategy for Containment of Antimicrobial Resistance recommendations 110

Appendix 2: List of 2011 WHO World Health Day six-point policy briefs 115

List of authors, contributors, reviewers and participants in consultation 117

IPC	Infection Prevention and Control
ISRAR	International Surveillance of Reservoirs of Antibiotic Resistance
LED	Light Emitting Diode
MDR	Multi-drug Resistance
MDR-TB	Multidrug-resistant Tuberculosis
MoH	Ministry of Health
MRSA	Methicillin-resistant Staphylococcus aureus
MSSA	Methicillin-sensitive Staphylococcus aureus
MYSTIC	Meropenem Yearly Susceptibility Test Information Collection
NAUSP	National Antimicrobial Utilization Surveillance Program
NCATS	National Center for Advancing Translational Sciences
NDM	New Delhi Metallo-beta-lactamase
NEQAS	National External Quality Assurance Scheme
NGOs	Nongovernmental Organizations
NIH	National Institutes of Health
OIE	World Organisation for Animal Health
OSDD	Open Source Drug Discovery
OTC	Over the Counter
PAHO	Pan American Health Organization
PATH	Program for Appropriate Technology in Health
PCR	Polymerase Chain Reaction
PDPs	Product Development Partnerships
PPS	Point Prevalence Survey
R&D	Research and Development
ReAct	Action on Antibiotic Resistance
ReLAVRA	Red Latinoamericana de Vigilancia a las Resistencias Antimicrobianas
SAR	Self-medication with Antibiotics and Resistance Levels in Europe
SEAR	South-East Asia Region
SIDA	Swedish International Development Cooperation Agency
SRL	Staphylococcus Reference Laboratory
STRAMA	Swedish Strategic Programme against Antibiotic Resistance
TATFAR	Transatlantic Task Force on Antimicrobial Resistance
TDR	Research and Training in Tropical Diseases
TPP	Target Product Profile
UMC	Uppsala Monitoring Centre
UNITAID	International facility for the purchase of drugs against HIV/AIDS, Malaria and Tuberculosis
VRE	Vancomycin-resistant enterococci
WHD	World Health Day
WHO	World Health Organization
WPR	Western Pacific Region
WPRO	WHO Regional Office for the Western Pacific
XDR-TB	Extensively Drug-resistance Tuberculosis

Abbreviations

AGISAR	WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance
AMC	Advanced Market Commitment
AMR	Antimicrobial Resistance
ANDI	African Network for Drugs and Diagnostics Innovation
ANSORP	Asian Network of Surveillance of Resistant Pathogens
APUA	Alliance for the Prudent Use of Antibiotics
ARMed	Antimicrobial Resistance in the Mediterranean
CDC	Centers for Disease Control
CIPARS	Canadian Integrated Programme for Antimicrobial Resistance Surveillance
CLSI	Clinical and Laboratory Standards Institute
CNISP	Canadian Nosocomial Infection Surveillance Programme
CSIR	Council of Scientific and Industrial Research
DDD	Defined Daily Doses
DID	Daily Doses per 1000 inhabitants per day
DRA	Drug Regulatory Agencies
DRG	Diagnosis Related Group
EARS-Net	European Antimicrobial Resistance Surveillance Network
ECDC	European Centre for Disease Prevention and Control
EML	Essential Medicines List
EMRO	WHO Regional Office for the Eastern Mediterranean
EQA	External Quality Assurance
ESAC-Net	European Surveillance of Antimicrobial Consumption Network
EUCAST	European Committee on Antimicrobial Susceptibility Testing
EUR	European Region
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FIND	Foundation for Innovative New Diagnostics
GAVI	Global Alliance for Vaccines and Immunization
GFN	Global Foodborne Infections Network
GLI	Global Laboratory Initiative
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis and Critical Control Point
HAI	Health care-Associated Infection
HELICS	Hospitals in Europe Linked for Infection Control through Surveillance
HHCCP	Hand Hygiene Culture-change Pilot Programme
HIV	Human Immunodeficiency Virus
HIVResNet	Global HIV Drug Resistance Network
HPV	Human Papilloma Virus
ICSRs	Individual Case Safety Reports
IDSR	Integrated Disease Surveillance and Response
IMI	Innovative Medicines Initiative
IMS	Intercontinental Marketing Services
INICC	International Nosocomial Infection Control Consortium

Adelaide, South Australia), David Gordon (Flinders Medical Centre, Bedford Park, South Australia), Joy Gregory (Department of Human Services, Melbourne, Victoria), Geoff Hogg (Microbiological Diagnostic Unit, University of Melbourne, Parkville, Victoria), Tim Inglis (Division of Microbiology & Infectious Diseases, PathWest, Nedlands, Western Australia), Peter Jelfs (Institute of Clinical Pathology and Medical Research, Westmead, New South Wales), Martyn Kirk (OzFoodNet, Canberra, Australian Capital Territory), Karin Lalor (Department of Human Services, Melbourne, Victoria), Jan Lanser (Institute of Clinical Pathology and Medical Research, Westmead, New South Wales), Lance Mickan (Institute of Medical and Veterinary Science, Adelaide, South Australia), Lyn O'Reilly (Division of Microbiology & Infectious Diseases, PathWest, Nedlands, Western Australia), Rosa Rios (Microbiological Diagnostic Unit, Parkville, Victoria), Minda Sarna (Department of Health, Perth, Western Australia), Hemant Sharma (Hunter New England Health Service, Newcastle New South Wales), Helen Smith (Queensland Health Scientific Services, Coopers Plains, Queensland), Leanne Unicomb (OzFoodNet, Hunter New England Population Health and National Centre for Epidemiology and Population Health, Australian National University, Canberra, Australian Capital Territory), and Mary Valcanis (Microbiological Diagnostic Unit, University of Melbourne, Parkville, Victoria).

Acknowledgments

Financial support. The OzFoodNet program of work (an initiative of the Australian Government Department of Health and Ageing) and New South Wales Health (through the Hunter Medical Research Institute).

Potential conflicts of interest. All authors: no conflicts.

References

1. Miller M, Roche P, Yohannes K, et al. Australia's notifiable disease status 2003: annual report or the National Notifiable Diseases Surveillance System. Commun Dis Intell **2005**; 29:1–61.
2. Nachamkin I, Ung H, Ming I. Increasing fluoroquinolone resistance in *Campylobacter jejuni*, Pennsylvania, USA, 1982–2001. Emerg Infect Dis **2002**; 8:1501–3.
3. The Campylobacter Sentinel Surveillance Scheme Collaborators. Ciprofloxacin resistance in *Campylobacter jejuni:* case-case analysis as a tool for elucidating risks at home and abroad. J Antimicrob Chemother **2002**; 50:561–8.
4. Hall GV, Kirk MD, Becker N, et al. Estimating foodborne gastroenteritis, Australia. The OzFoodNet Working Group. Emerg Infect Dis **2005**; 11:1257–64.
5. Engberg J, Aarestrup FM, Taylor DE, Gerner-Smidt P, Nachamkin I. Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli*: resistance mechanisms and trends in human isolates. Emerg Infect Dis **2001**; 7:24–34.
6. Travers K, Barza M. Morbidity of infections caused by antimicrobial-resistant bacteria. Clin Infect Dis **2002**; 34 (Suppl 3):S131–4.
7. Nelson JM, Smith KE, Vugia DJ, et al. Prolonged diarrhea due to ciprofloxacin-resistant *Campylobacter* infection. J Infect Dis **2004**; 190:1150–7.
8. Helms M, Simonsen J, Olsen KEP, Molbak K. Adverse health events associated with antimicrobial drug resistance in *Campylobacter* species: a registry-based cohort study. J Infect Dis **2005**; 191:1050–5.
9. Bywater R, Deluyker H, Deroover E, et al. A European survey of antimicrobial susceptibility among zoonotic and commensal bacteria isolated from food-producing animals. J Antimicrob Chemother **2004**; 54:744–54.
10. Sharma H, Unicomb L, Forbes W, Djordjevic S, Valcanis M, Dalton C, Ferguson J. Antibiotic resistance in *Campylobacter jejuni* isolated from humans in the Hunter Region, New South Wales. Commun Dis Intell **2003**; 27 Suppl:S80–8.
11. Alfredson DA, Akhurst RJ, Korolik V. Antimicrobial resistance and genomic screening of clinical isolates of thermophilic *Campylobacter* spp. from south-east Queensland, Australia. J Appl Microbiol **2003**; 94:495–500.
12. Unicomb L, Ferguson J, Riley T, Collignon P. Absence of fluoroquinolone resistance among *Campylobacter* isolates from humans in Australia. Emerg Infect Dis **2003**; 9:1482–3.
13. Linton D, Lawson AJ, Owen RJ, Stanley J. PCR detection, identification to species level, and fingerprinting of *Campylobacter jejuni* and *Campylobacter coli* direct from diarrhoeic samples. J Clin Microbiol **1997**; 35:2568–72.
14. National Committee for Clinical Laboratory Standards (NCCLS). Performance standards for antimicrobial susceptibility testing: fourteenth informational supplement. NCCLS document M100-S14. Wayne, Pennsylvania: NCCLS, **2004**.
15. Euzéby JP. List of bacterial names with standing in nomenclature: a folder available on the internet. Int J Systematic Bacteriol **1997**; 47: 590–2.
16. National Notifiable Diseases Database. Available at: http://www1.health.gov.au/cda/Source/CDA-index.cfm. Accessed 14 July 2005.
17. Hart WS, Heuzenroeder MW, Barton MD. Antimicrobial resistance in *Campylobacter* spp., *Escherichia coli* and enterococci associated with pigs in Australia. J Vet Med B Infect Dis Vet Public Health **2004**; 51:216–21.
18. Osterlund A, Hermann M, Kahlmeter G. Antibiotic resistance among *Campylobacter jejuni/coli* strains acquired in Sweden and abroad: a longitudinal study. Scand J Infect Dis **2003**; 35:478–81.
19. Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP). DANMAP 2004: use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark. DANMAP, **2005**. Available at: http://www.dfvf.dk/files/filer/zoonosecentret/publikationer/danmap/danmap_2004.pdf. Accessed 10 April 2006.
20. Tjaniadi P, Lesmana M, Subekti D, et al. Antimicrobial resistance of bacterial pathogens associated with diarrheal patients in Indonesia. Am J Trop Med Hyg **2003**; 68:666–70.
21. Heymann DL, ed. Control of communicable diseases manual. 18th ed. Washington D.C., American Public Health Association Press, **2004**.
22. Smith KE, Besser JM, Hedberg CW, et al. Quinolone-resistant *Campylobacter jejuni* infections in Minnesota, 1992–1998. N Engl J Med **1999**; 340:1525–32.
23. Thwaites RT, Frost JA. Drug resistance in *Campylobacter jejuni, C. coli* and *C. lari* isolates from humans in North West England and Wales, 1997. J Clin Pathol **1999**; 52:812–4.
24. Huysmans MB, Turnidge JD. Disc susceptibility testing for thermophilic *Campylobacters.* Pathology **1997**; 29:209–16.
25. Gupta A, Nelson JM, Barrett TJ, et al. Antimicrobial resistance among *Campylobacter* strains, United States, 1997–2001. The NARMS Working Group. Emerg Infect Dis **2004**; 10:1102–9.
26. Talsma E, Goettsch WG, Nieste HLJ, Schrijnemakers PM, Sprenger MJW. Resistance in *Campylobacter* species: increased resistance to fluoroquinolones and seasonal variation. Clin Infect Dis **1999**; 29: 845–8.

in those regions. These regions include countries in which Australian travellers are likely to acquire infection; for example, >80% of clinical isolates in Thailand have been reported to be ciprofloxacin resistant [5], and 35% of clinical isolates in Indonesia were ciprofloxacin resistant [20].

The source of resistant isolates from locally acquired infections is unclear. It is possible that the case-patient had been overseas >7 days before the onset of illness or had direct contact with a recently returned traveller; however, person-to-person transmission is uncommon [21]. Other possible sources include consumption of contaminated, imported food or acquisition of resistance during a hospital stay. However, only 2 of the 14 patients infected with ciprofloxacin-resistant isolates had been admitted to a hospital for treatment. Furthermore, only cooked chicken is permitted for importation into Australia [12], and viable *Campylobacter* organisms are unlikely to be present.

Previous studies have shown that treatment of patients with quinolones after onset of illness but before collection of the stool specimen is associated with detection of quinolone-resistant isolates [22]. We did not collect information on the timing of antimicrobial treatment or the agent used. However, patients with ciprofloxacin-resistant isolates were significantly more likely to have been given antibiotic therapy for their infection than were patients infected with susceptible isolates, and ciprofloxacin is commonly used in Australia for the treatment of enteric infections. Therefore, this explanation is plausible.

Three previous Australian studies that reported travel histories of patients examined 153, 140, and 50 isolates, respectively [12]; the sample sizes in these studies may have been insufficient to detect a low prevalence of resistant isolates. The reasons for variation in the apparent prevalence of locally acquired ciprofloxacin-resistant isolates by state of residence are unclear and require further study.

Resistance to sulfisoxazole, ampicillin, and roxithromycin was detected commonly among isolates obtained from patients that acquired their infections locally. The prevalence of ampicillin resistance in this study (46%) was similar to that reported in previous Australian studies [10, 11] and studies from the United Kingdom [23]. Low levels of resistance were detected for erythromycin, chloramphenicol, gentamicin, and kanamycin, as reported in Australia [10, 24] and elsewhere [18, 19, 23, 25]. Tetracycline resistance was low, which contrasts with findings from the United States [25] but is similar to findings from Europe [18, 19, 26]. Isolates from travel-associated cases were more commonly resistant to nalidixic acid, ciprofloxacin, and tetracycline than were isolates from locally acquired cases, as found previously in Australia [10] and Denmark [19]. Isolates resistant to nalidixic acid and susceptible to ciprofloxacin were found among travel-acquired and locally acquired isolates, as found in other studies [10, 23]. Six percent of case-patients had taken an antimicrobial agent in the 4 weeks before onset of illness, but there was no association between this and ciprofloxacin resistance.

In our study, infection with a ciprofloxacin-resistant isolate was not associated with increased severity of illness; this contrasts with a study from the United States, in which a similar number of patients infected with ciprofloxacin-resistant organisms was more likely to be hospitalized and have bloody diarrhea [25] than were patients infected with susceptible isolates. A study [7] that described patients infected with ciprofloxacin-resistant isolates who had prolonged diarrhea included 63 patients; it is possible that the larger sample size raised the statistical power of the study to detect a difference.

There are some limitations in generalizing the results of this study to the Australian population. First, patients included in this study were identified through notifications and were, therefore, more likely to have had relatively severe infections, leading to presentation and a stool test. Second, we relied on self-reported information regarding overseas travel, antimicrobial therapy, and clinical symptoms, and this information was not validated. However, the potential measurement bias resulting from this method was likely to be nondifferential (i.e., occurring equally among patients infected with resistant and susceptible strains).

In summary, antimicrobial resistance among Australian strains of *C. jejuni* is uncommon, excepting resistance to ampicillin, roxithromycin, and sulfisoxazole. Of particular importance is that resistance to fluoroquinolone is very low and probably reflects Australia's policy of prohibiting fluoroquinolones for animal use. Sensible use of fluoroquinolones in clinical treatment remains a high priority if a low prevalence of resistance in *C. jejuni* and other organisms is to be maintained. The United States withdrew approval for the use of fluoroquinolones in animals following reports between 1994 and 1996 of the increasing levels of fluoroquinolone resistance. Such a policy should be considered for wider adoption. The detection of ciprofloxacin resistance among locally acquired infections warrants additional investigations and ongoing surveillance.

AUSTRALIAN CAMPYLOBACTER SUBTYPING STUDY GROUP

Penny Adamson (Flinders Medical Centre, South Australia), Kellie Cheung (Institute of Clinical Pathology and Medical Research, Westmead, New South Wales), Barry Combs (Department of Human Services, Adelaide, South Australia), Craig Dalton (Hunter New England Population Health, Newcastle, New South Wales), Steve Djordjevic (Elizabeth Macarthur Agricultural Institute, Camden, New South Wales), Robyn Doyle (Institute of Medical and Veterinary Science, Adelaide, South Australia), John Ferguson (Hunter New England Health Service, Newcastle, New South Wales), Lyn Gilbert (Institute of Clinical Pathology and Medical Research, Westmead, New South Wales), Rod Givney (Department of Human Services,

Table 3. Characteristics of patients infected with locally-acquired ciprofloxacin-resistant *Campylobacter jejuni*.

	Patient characteristics						Isolate characteristics	
Patient no., by state	Age, years	Sex	Date of onset	Underlying disease	Antimicrobial agent included in treatment[a]	Antimicrobial agent taken 4 weeks prior to onset of illness	MIC for ciprofloxacin, mg/L	Resistance to other antimicrobial agents
Victoria								
31	39	Female	18 Nov 2001	...	Yes	...	32	Nalidixic acid, roxithromycin, sulfisoxazole
44[b]	36	Female	8 Nov 2001	Diabetes	No	...	32	Nalidixic acid, tetracyline, ampicillin, gentamicin, kanamycin, sulfisoxazole
196	20	Male	15 Dec 2001	...	Yes	...	16	Nalidixic acid, sulfisoxazole
354	61	Female	21 Jan 2002	Arthritis	No	...	16	Nalidixic acid, sulfisoxazole
360	78	Female	26 Jan 2002	Hypertension, arthritis	Yes	Roxithromycin	16	Nalidixic acid, chloramphenicol
383	59	Female	6 Feb 2002	Hypertension, arthritis	Yes	...	16	Nalidixic acid, chloramphenicol
401[b]	83	Female	12 Feb 2002	Diabetes, arthritis	Yes	...	16	Nalidixic acid
674	65	Male	9 Apr 2002	arthritis	Yes	...	16	Nalidixic acid, sulfisoxazole
Queensland								
1187	66	Male	30 Jul 2002	Hypertension	No	...	32	Nalidixic acid, sulfisoxazole
South Australia								
116	23	Female	4 May 2002	...	No	...	64	Nalidixic acid, ampicillin, roxithromycin
174	80	Female	24 May 2002	Arthritis	No	...	32	Nalidixic acid
1700	64	Male	24 May 2002	Diabetes, liver disease, arthritis	Yes	...	32	Nalidixic acid
4430	14	Female	10 Jul 2002	...	Yes	...	32	Nalidixic acid
Western Australia								
90	2	Female	25 Apr 2002	NA	Yes	...	8	Nalidixic acid, ampicillin, erythromycin, roxithromycin, gentamicin

NOTE. NA, not available.

[a] Names of antimicrobial agents were not recorded.

[b] Patient was admitted to a hospital.

Table 1. Summary of patients with *Campylobacter* infection and the *Campylobacter jejuni* isolates included in the study.

State[a]	Population (in millions)[b]	Recruitment period	No. of *Campylobacter* infections reported during the recruitment period[c]	No. of patients with *Campylobacter* infections	No. of *C. jejuni* isolates tested[d]	Isolates obtained from reported patients that were tested, %	No. of travel-acquired isolates tested
Queensland	3.51	February–August 2002	1847	215	117	6.3	3
South Australia	1.49	February–August 2002	1244	242	177	14.2	0
Tasmania	0.47	September 2001–August 2002	619	199	62	10.0	3
Victoria	4.70	October 2001–May 2002	3683	201	108	2.9	5
Western Australia	1.86	November 2001–August 2002	1824	160	121	6.6	0
Total	12.03	September 2001–August 2002	9217	1017	585	6.3	11

[a] All states that reported *Campylobacter* infections to the National Notifiable Diseases Database have been included in the study. Neither of the 2 territories were included.
[b] Population estimated from the 2001 census (Australia Bureau of Statistics).
[c] All *Campylobacter*-infected patients reported to National Notifiable Diseases Database [16].
[d] Species determined using PCR [13].

Table 2. Percentage of locally acquired *Campylobacter jejuni* isolates resistant to 10 antimicrobial agents by state, September 2001–August 2002.

State	No. of isolates tested	Resistant isolates, %[a]									
		Nalidixic acid	Ciprofloxacin	Tetracycline	Ampicillin	Erythromycin	Roxithromycin	Gentamicin	Kanamycin	Chloramphenicol	Sulfisoxazole
Queensland	114	4.4	0.9	14.9	54.4	0.9	53.5	0.0	0.0	0.9	86.0
South Australia	177	5.6	2.3	6.8	45.8	2.3	41.8	0.0	0.0	0.0	37.9
Tasmania	59	0.0	0.0	1.7	39.0	0.0	30.5	0.0	0.0	0.0	71.2
Victoria	103	8.7	7.8	2.9	44.7	1.0	51.5	1.0	1.0	6.8	74.8
Western Australia	121	9.1	0.8	4.1	43.0	7.4	9.1	6.6	0.0	5.8	27.3
National	574	6.1	2.4	6.6	46.0	2.6	37.8	1.6	0.2	2.6	55.2

[a] Percentage of all isolates tested for resistance from the respective state that were resistant to the specified antimicrobial agent.

RESULTS

The characteristics of the study population, including the number of cases of *Campylobacter* infection reported during the study period, the number of case-patients recruited, and the proportion of isolates tested, are summarized by state in table 1. Of the 585 isolates tested, 279 (48%) were from female patients, and the median age was 32 years (range, 0–93 years). The proportion of *Campylobacter* isolates tested for antimicrobial susceptibility from reported case-patients from each state ranged from 3% in Victoria to 14% in South Australia. The population under surveillance in the 5 participating states comprised ~64% of the Australian population in 2001.

Prevalences of resistance to the 10 antimicrobial agents among locally acquired isolates is shown in table 2. Sulfisoxazole resistance was the most common (55% of isolates), and only 2% of isolates were resistant to ciprofloxacin.

Ciprofloxacin resistance was found among locally acquired isolates from all states except Tasmania (table 2). All 14 locally acquired isolates were also resistant to nalidixic acid, and 10 (71%) were resistant to >1 class of antimicrobial agent (table 3). Temporal clusters of infection were detected in Victoria (November–December 2001 and January–February 2002) and in South Australia (May 2002), but a variety of resistance phenotypes were detected in these clusters, indicating that the isolates were unlikely to be related (table 3). The prevalence of ciprofloxacin resistance in Victoria was higher than in all other states combined (9% vs. 2%; OR, 6.2; 95% CI, 2.1–18.5), but this significant difference should be interpreted with care, because it is a post hoc comparison. When controlling for jurisdiction, patients infected with ciprofloxacin-resistant isolates were more likely to have an underlying disease than those infected with ciprofloxacin-susceptible isolates (OR 5.1; 95% CI, 1.5–17.3).

People who acquired *Campylobacter* infections overseas were more likely to be infected with resistant strains; 9 (82%) of 11 overseas-acquired isolates were resistant to >1 class of antimicrobial agent, compared with 291 (51%) of 574 locally acquired isolates (OR, 4.4; 95% CI, 0.9–41.9). Resistances to ciprofloxacin and tetracycline were significantly more prevalent among overseas-acquired isolates than they were among locally acquired isolates: ciprofloxacin, 64% vs. 2% (OR, 67.5; 95% CI, 15.2–351.6); and tetracyline, 55% vs. 7% (OR, 16.7; 95% CI, 4.0–72.6). Nine of 11 patients had travelled to Asia (Indonesia, Malaysia, Singapore, Thailand, and Vietnam) during the week before onset of illness; the remaining 2 patients had travelled to Africa and North and South America (data not shown).

Patients infected with locally acquired ciprofloxacin-resistant strains were no more likely to have taken an antimicrobial agent in the 4 weeks before onset, compared with patients infected with ciprofloxacin-susceptible isolates (6% vs. 7%; OR, 1.3; 95% CI, 0.2–10.2). No patient infected with a ciprofloxacin-resistant isolate had taken a fluoroquinolone in the 4 weeks before onset of illness. Nine (64%) of 14 patients with ciprofloxacin-resistant isolates were given antibiotic therapy for their *Campylobacter* infection, compared with 222 (40%) of 549 patients with ciprofloxacin-susceptible isolates; however, this difference did not reach statistical significance (OR, 2.5; 95% CI, 0.9–7.1).

Infection with a ciprofloxacin-resistant strain of *C. jejuni* did not result in a more-severe illness, but the number of patients that were infected with a ciprofloxacin-resistant strain of *C. jejuni* was small. There were no significant differences in the distribution of symptoms; compared with patients infected ciprofloxacin-susceptible strains, patients infected with ciprofloxacin-resistant strains were no more likely to have fever (68% vs. 74%; OR, 1.6; 95% CI, 0.4–5.9), or vomiting (38% vs. 35%; OR, 1.6; 95% CI, 0.6–4.5), or bloody stools (15% vs. 42%; OR, 0.4; 95% CI, 0.1–5.4). Duration of diarrhea was similar for patients infected with ciprofloxacin-resistant strains and patients infected with ciprofloxacin-sensitive strains (median duration for both groups, 7 days; $P = .63$), as were the percentage of patients requiring hospitalization (14% vs. 13%; OR, 0.8; 95% CI, 0.2–3.6) and the length of hospital stay (median duration for both groups, 0 days; $P = .13$) in multivariate models controlling for age and underlying disease, regardless of travel status.

DISCUSSION

This is the first Australian study to report locally acquired *Campylobacter* isolates resistant to fluoroquinolones. However, the prevalence of ciprofloxacin resistance among locally acquired isolates in Australia was low at 2% and ranged from 0% to 8% across 5 states. The absence of ciprofloxacin-resistant isolates in locally acquired infections in Australia has been attributed previously to restricting the use of fluoroquinolones in food-producing animals. Data regarding antimicrobial susceptibility among *Campylobacter* isolates infecting Australian food-producing animals is limited; however, *Campylobacter* isolates (all species) from pigs have been shown to be uniformly susceptible to ciprofloxacin [17], likely reflecting the low prevalence of resistance in isolates obtained from Australian animals. The prevalence is similar to that described for humans from Sweden (between 0% and 9%), where the use of antibiotics as growth promoters was banned in 1986 [18]. A low prevalence of ciprofloxacin resistance was also found among isolates obtained from Swedish animals [9]. Among studies that have separated locally acquired from travel-acquired isolates, in countries that have allowed the use of fluoroquinolones for animals, the prevalence of locally acquired ciprofloxacin resistance ranges from 7% to 29% [3, 19]. The overall prevalence of ciprofloxacin resistance is much higher in some countries, probably reflecting widespread use of ciprofloxacin in humans

Key findings of Consumer Reports research are:

1. In a recent nationwide poll conducted by the Consumer Reports National Research Center, 86 percent of consumers indicated they thought that meat raised without antibiotics should be available in their local supermarket.

2. Consumer Reports shoppers visited 136 supermarkets in 23 states, including at least five stores belonging to each of the 13 largest (by sales) supermarket chains in the nation, and collected data on more than 1,000 different meat and poultry items making some type of "no antibiotics" claim on a label. The shoppers found wide geographic availability, but big differences among chains and stores in availability of meat and poultry raised without antibiotics. On the one hand, Whole Foods guarantees that all meat and poultry sold in its stores is never treated with antibiotics. Shoppers also found wide selections of meat and poultry raised without antibiotics at Giant, Hannaford, Shaw's, and Stop & Shop. At the other extreme, shoppers at Sam's Club, Food 4 Less, Food Lion, and Save-A-Lot stores could not find any meat or poultry indicating it was raised without antibiotics.

3. In the Consumer Reports poll, 24 percent of consumers said meat raised without antibiotics was not available at the supermarket where they usually shop. Of this group, 82 percent said they would buy it if it were available.

4. Meat and poultry raised without antibiotics does not have to be expensive. While prices of such meat and poultry varied considerably depending on store, type of meat (beef, pork, chicken, turkey) and cut, in some cases our shoppers found prices that were actually lower than the national average. For example, while the national average price in March 2012 for chicken breasts was $3.17 per pound, our shoppers found chicken breasts produced without antibiotics at QFC for $2.99 per pound and on sale at Whole Foods for $1.99 per pound. The most expensive product raised without antibiotics that Consumer Reports shoppers spotted was organic ribeye steak for $19.99 per pound at several Kroger stores. However, much cheaper products were also widely available. The least expensive no antibiotics products were whole chickens at Publix and Jewel-Osco, and chicken drumsticks at several Trader Joe's locations, all for $1.29 per pound.

5. Studies over the last decade have indicated that raising meat and poultry without antibiotics could be accomplished at minimal cost to the consumer—about 5 cents extra per pound for pork and less than a penny per pound extra for chicken. In the Consumer Reports survey, 61 percent of consumers indicated they would pay 5 cents or more extra per pound, and 37 percent indicated they would pay $1.00 a pound or more extra for meat and poultry raised without antibiotics.

6. Consumer Reports shoppers found a wide array of labels related to antibiotic use, such as "never ever given antibiotics," "humanely raised on family farms without antibiotics," "organic," and "grassfed." Consumer Reports analyzed the various labels and concluded that most of them are at least somewhat useful to consumers. Consumers can always rely on the "organic" label, since organic rules ban antibiotic use in livestock. In addition, consumers can generally rely on most labels that contain the words "no antibiotics" or "raised without antibiotics" especially if it is "USDA Process Verified" (meaning that the USDA has checked up to see whether the producer is actually doing what it claims).

But Consumer Reports shoppers found a few labels that consumers should not rely upon as indicators that a product has truly had no antibiotics throughout the growing process. They include "natural," "antibiotic-free," "no antibiotic residues," and "no antibiotic growth promotants." "Natural" means only that the product contains no artificial ingredient or added color and is only minimally processed, according to the USDA. Antibiotics can in fact be used in the raising of "natural" meat and poultry. The terms "antibiotic-free" and "no antibiotic residues" are terms that the USDA does not approve for use on meat and poultry, so their meaning is uncertain, and they should not appear in the marketplace. The label "no antibiotic growth promotants," also

Executive Summary

The declining effectiveness of antibiotics has become a major national public health crisis. According to the national Centers for Disease Control and Prevention, 99,000 people died of hospital-acquired infections in 2002, the most recent year for which data are available. According to the Infectious Diseases Society of America, the vast majority of those infections were caused by antibiotic-resistant bacteria. Such "superbugs"—bacteria resistant to one or more antibiotics—are also showing up in food and causing illness and even death. Doctors and scientists have called for much more careful use of antibiotics so that disease-causing organisms don't become immune to them.

The major user of antibiotics in the United States today is not the medical profession, however, but the meat and poultry business. Some 80 percent of all antibiotics sold in the United States are used not on people but on animals, to make them grow faster or to prevent disease in crowded and unsanitary conditions. Consumers Union, the advocacy arm of Consumer Reports, believes that to preserve antibiotics for treatment of disease in people, use on animals must be drastically reduced or eliminated.

A key question is how this can be accomplished. Many groups and experts have urged the U.S. Food and Drug Administration (FDA) and Congress to ban the use of antibiotics in animal feed. But the pharmaceutical industry and large-scale livestock producers, which benefit economically from their use, have effectively opposed all such proposals for decades.

Supermarkets and consumers, however, have a major say about antibiotic use in animals through their purchasing decisions. Although antibiotics remain legal to use on food animals, supermarkets can choose not to carry, and consumers can choose not to buy, meat and poultry from animals that are fed antibiotics. The vast majority of all meat and poultry produced in the United States is either sold to consumers in supermarkets and grocery stores or consumed in restaurants and schools and other institutions. (The remainder, about 15 percent, is exported.) The purchasing decisions that supermarkets and consumers make therefore have a profound effect on how food animals are raised.

Consumer Reports has undertaken this report to determine what consumers think about reducing antibiotic use in meat and poultry production, and whether major supermarkets are making products that are raised without antibiotics available to their customers. We polled consumers, contacted companies, and sent shoppers into stores to find out.

The news is encouraging. At least one of the 13 largest supermarket chains in the country, Whole Foods, offers nothing but meat and poultry raised without antibiotics in its meat department. Most other major chains offer some such products. And the prices are not prohibitive—a number of supermarkets are offering chicken without antibiotics at $1.29 a pound, for example, a price that is competitive with all chicken prices nationally. Other studies suggest that pork raised without antibiotics should cost less than 5 cents a pound extra.

of antimicrobial resistance among Australian isolates of *Campylobacter jejuni*. Australia is in an almost unique position in that it is has prohibited fluoroquinolones from being used in food-producing animals, although it has animal production and food production systems comparable to those of other developed nations. By measuring the prevalence of fluoroquinolone resistance among *C. jejuni* isolates obtained from Australian patients, some insight might be gained into the benefit of stricter control over the use of medically important antimicrobials in food animals. Prevalence of resistance was examined for *Campylobacter* isolates obtained from patients from 5 jurisdictions, representing ~60% of the Australian population, over a 1-year period and was compared by travel status and jurisdiction.

METHODS

Study population. *Campylobacter* isolates were collected from case-patients enrolled in a multicenter, prospective, case-control study of sporadic infection (to be reported separately). The case-patients were identified from laboratory reports from 5 Australian states (Queensland, South Australia, Tasmania, Victoria, and Western Australia) between September 2001 and August 2002. These jurisdictions represented all states that require doctors and laboratories to report patients infected with *Campylobacter* species. The 2 Australian territories were not included, and New South Wales, where *Campylobacter* is not notifiable, was not included. Each jurisdiction aimed to recruit ~200 patients of all ages using a systematic method of selection. Patients were excluded if they could not be contacted, their parents were not English speakers, they could not answer questions (e.g., because of dementia or because they were deceased), they could not recall the date of onset of their diarrhea, onset was ⩾10 days before the specimen was collected, they could not be interviewed within 30 days after onset, another member of the household had had diarrhea or had been diagnosed with *Campylobacter* infection in the previous 4 weeks, they had a mixed infection (i.e., an additional diarrheal pathogen was simultaneously detected), they were part of an outbreak, or they refused consent.

A telephone-administered questionnaire was used to document exposures for the 7 days before onset of illness. Questions included details of overseas travel (country visited and travel dates), demographic characteristics (age and sex), severity of illness (duration of diarrhea, bloody stools, fever, and vomiting), care management (hospital admission and duration of hospitalization), consumption of antimicrobial agents in the 4 weeks before onset, and underlying diseases.

Laboratory methods. The *Campylobacter* isolates were transported from the clinical laboratory to the state public health laboratory for storage and additional testing. *C. jejuni* isolates were distinguished from non-*jejuni* species at each public health laboratory using PCR that targeted the *Hip*O gene [13], and only *C. jejuni* isolates (subspecies *jejuni* or *doylei*) were included in the study.

Susceptibility testing was performed at each public health laboratory by the agar dilution method using Mueller-Hinton agar with 5% lysed sheep blood, in accordance with National Committee for Clinical Laboratory Standards (now called Clinical and Laboratory Standards Institute [CLSI]), as described elsewhere [14]. MICs were defined as the lowest concentration giving complete inhibition of visible growth. Antimicrobial agents tested and breakpoints denoting resistance were as follows: nalidixic acid, ⩾32 mg/L; ciprofloxacin, ⩾4 mg/L; tetracycline, ⩾16 mg/L; ampicillin, ⩾32 mg/L; erythromycin, ⩾8 mg/L; roxithromycin, ⩾8 mg/L; gentamicin, ⩾8 mg/L; kanamycin, ⩾32 mg/L; chloramphenicol, ⩾32 mg/L; and sulfisoxazole, ⩾ 350 mg/L. Because there are no recommended breakpoints specifically for *Campylobacter* species, the CLSI breakpoints for *Enterobacteriaceae* were used, except for erythromycin, for which the breakpoint for *Staphylococcus* was used [14].

Before the study began, a set of 8 isolates that had been previously tested for susceptibility to 7 of the 10 agents used in the study [10] were tested by each laboratory to ensure reproducibility of results. During the testing of study isolates, *C. jejuni* NCTC 11351 (same isolate as the CLSI-recommended ATCC 33560 [15]) was included as the control in each test batch.

Statistical analyses. We compared the following: (1) patients infected with ciprofloxacin-resistant isolates and patients infected with susceptible isolates; (2) the prevalence of resistance among locally acquired isolates and the prevalence among isolates acquired overseas; and (3) the severity of disease among patients infected with ciprofloxacin-susceptible *Campylobacter* and the severity of disease among patients infected with ciprofloxacin-resistant *Campylobacter*. The following severity indicators were examined: duration of diarrhea, presence of blood in the stool, fever and vomiting, hospitalization, and length of hospital stay. The 95% CIs for prevalence and ORs were based on standard large sample methods for estimates of proportions. Exact methods were used when counts were small. In particular, Fisher's exact test was used to the compare prevalence of antimicrobial resistance in locally acquired isolates with the prevalence of resistance in travel-acquired isolates. Prevalences of resistance among isolates from locally acquired infections were compared across jurisdictions and by antimicrobial exposure status using likelihood ratio tests based on logistic regression, with potential confounders included in the analyses.

Logistic regression was also used to compare dichotomous measures of severity of illness, such as hospitalization and blood in stool, whereas standard linear regression was used to compare continuous measures, such as duration of illness and length of hospital stay. Statistical analyses were performed using STATA, version 9.1 (Stata).

MAJOR ARTICLE

Low-Level Fluoroquinolone Resistance among *Campylobacter jejuni* Isolates in Australia

Leanne E. Unicomb,[2,7] John Ferguson,[3,4] Russell J. Stafford,[1] Rosie Ashbolt,[6] Martyn D. Kirk,[8] Niels G. Becker,[7] Mahomed S. Patel,[7] Gwendolyn L. Gilbert,[8] Mary Valcanis,[9] and Lance Mickan,[10] for the Australian Campylobacter Subtyping Study Group[a]

[1]OzFoodNet, Queensland Health, Archerfield, Queensland, [2]OzFoodNet, Hunter New England Population Health, Wallsend, [3]Hunter New England Health Service, Newcastle, [4]University of Newcastle, Callaghan, and [5]Centre for Infectious Diseases & Microbiology, Institute of Clinical Pathology and Medical Research, Wentworthville, New South Wales, [6]OzFoodNet, Department of Health and Human Service, Hobart, Tasmania, [7]National Centre for Epidemiology and Population Health, Australia National University, and [8]OzFoodNet, Department of Health and Ageing, Canberra, Australian Capital Territory, [9]Microbiological Diagnostic Unit, Department of Microbiology and Immunology, University of Melbourne, Parkville, Victoria, and [10]Institute of Medical and Veterinary Sciences, Rundle Mall, Adelaide, South Australia, Australia

Background. Ciprofloxacin-resistant *Campylobacter jejuni* isolates obtained from infected patients in Australia have not been detected in studies of isolates from specific geographic areas. The Australian government has prohibited the use of fluoroquinolone in food-producing animals. To assess the impact of this policy, we have examined the antimicrobial susceptibility of isolates from 5 Australian states.

Methods. We conducted a period-prevalence survey of the susceptibility of *C. jejuni* isolates to 10 antimicrobial agents. *C. jejuni* isolates obtained from 585 patients from 5 Australian states (Queensland, South Australia, Tasmania, Victoria, and Western Australia) were identified by means of notifiable disease databases and were systematically selected from September 2001 to August 2002.

Results. Among locally acquired infections, only 2% of isolates (range, 0%–8% in different states) were resistant to ciprofloxacin. The locally acquired isolates also exhibited resistance to sulfisoxazole (55%), ampicillin (46%), roxithromycin (38%), tetracycline (7%), nalidixic acid (6%), chloramphenicol (3%), erythromycin (3%), gentamicin (2%), and kanamycin (0.2%). Treatment with antimicrobial agents in the 4 weeks before onset was not associated with ciprofloxacin resistance.

Conclusions. The very low level of ciprofloxacin resistance in *C. jejuni* isolates likely reflects the success of Australia's policy of restricting use of fluoroquinolones in food-producing animals.

Campylobacter species are the most common bacterial cause of foodborne disease in Australia and other industrialized countries [1–3] and constitute a substantial health burden. The incidence of reported cases in Australia was 116.5 cases per 100,000 persons in 2003 [1], and ~277,000 cases of *Campylobacter* infection are estimated to occur annually [4].

In Europe and the United States, increasing proportions of patients are infected with strains of *Campylobacter* species exhibiting antimicrobial resistance, particularly resistance to fluoroquinolones [2, 5]. Antimicrobial resistance may add to the burden of disease; fluoroquinolone-resistant organisms have been reported to be associated with more-severe disease [6], including diarrhea of a longer duration [7] and an increased likelihood of invasive disease and death [8]. The rising incidence of fluoroquinolone resistance has been attributed to the use of fluoroquinolones in food-producing animals [5] and has been reflected in the high prevalence of ciprofloxacin-resistant animal *Campylobacter* isolates in animals in those countries [9].

Surveillance of antimicrobial resistance is important for monitoring trends. Data regarding antimicrobial resistance among *Campylobacter* isolates in Australia are limited, and studies have been confined to specific geographic regions [10–12]. Resistant isolates are not common, and fluoroquinolone resistance has not been detected previously among locally acquired isolates [12].

The aim of this study was to estimate the prevalence

Received 6 November 2005; accepted 23 January 2006; electronically published 13 April 2006.

[a] Members of the study group are listed at the end of the text.

Reprints or correspondence: Ms. Leanne Unicomb, National Centre for Epidemiology and Population Health, Australia National University, Canberra, ACT 0200, Australia (leanne.unicomb@anu.edu.au).

Clinical Infectious Diseases 2006; 42:1368–74

1058-4838/2006/4210-0004$15.00

2. Karisik E, Ellington MJ, Livermore DM *et al.* Virulence factors in extra-intestinal *Escherichia coli* with CTX-M β-lactamases in the United Kingdom. In: *Abstracts of the Forty-sixth Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, USA, 2006.* Abstract B1314, p. 50. American Society for Microbiology, Washington, DC, USA.

3. Livermore DM, Canton R, Gniadkowski M *et al.* CTX-M: changing the face of ESBLs in Europe. *J Antimicrob Chemother* 2007; **59**: 165–74.

4. Canton R, Coque TM. The CTX-M β-lactamase pandemic. *Curr Opin Microbiol* 2006; **9**: 1–10.

5. Bonnet R. Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. *Antimicrob Agents Chemother* 2004; **48**: 1–14.

6. Ensor VM, Shahid M, Evans JT *et al.* Occurrence, prevalence and genetic environment of CTX-M beta-lactamases in Enterobacteriaceae from Indian hospitals. *J Antimicrob Chemother* 2006; **58**: 1260–3.

7. Riano I, Moreno MA, Teshager T *et al.* Detection and characterization of extended-spectrum β-lactamases in *Salmonella enterica* strains of healthy food animals in Spain. *J Antimicrob Chemother* 2006; **58**: 844–7.

8. Hasman H, Mevius D, Veldman K *et al.* β-Lactamases among extended-spectrum β-lactamase (ESBL)-resistant *Salmonella* from poultry, poultry products and human patients in The Netherlands. *J Antimicrob Chemother* 2005; **56**: 115–21.

9. Kojima A, Ishii Y, Ishihara K *et al.* Extended-spectrum-β-lactamase-producing *Escherichia coli* strains isolated from farm animals from 1999 to 2002: report from the Japanese veterinary antimicrobial resistance monitoring program. *Antimicrob Agents Chemother* 2005; **49**: 3533–7.

10. Blanc V, Mesa R, Saco M *et al.* ESBL- and plasmidic class C β-lactamase-producing *E. coli* strains isolated from poultry, pig and rabbit farms. *Vet Microbiol* 2006; **118**: 299–304.

11. Duan RS, Sit TH, Wong SS *et al. Escherichia coli* producing CTX-M β-lactamases in food animals in Hong Kong. *Microb Drug Resist* 2006; **12**: 145–8.

12. Linton AH, Howe K, Bennett PM *et al.* The colonization of the human gut by antibiotic-resistant *Escherichia coli* from chickens. *J Appl Bacteriol* 1977; **43**: 465–9.

13. Gross WG. Diseases due to *Escherichia coli* in poultry. In: Gyles CL, ed. *Escherichia coli in Domestic Animals and Humans.* Wallingford, UK: CAB International, 1994; 237–59.

14. Rodriguez-Siek KE, Giddings CW, Doetkott C *et al.* Comparison of *Escherichia coli* isolates implicated in human urinary tract infection and avian colibacillosis. *Microbiology* 2005; **151**: 2097–110.

15. Wani SA, Samanta I, Bhat MA *et al.* Investigation of shiga toxin-producing *Escherichia coli* in avian species in India. *Lett Appl Microbiol* 2004; **39**: 389–94.

16. Johnson JR, Murray AC, Gajewski A *et al.* Isolation and molecular characterization of nalidixic acid-resistant extraintestinal pathogenic *Escherichia coli* from retail chicken products. *Antimicrob Agents Chemother* 2003; **47**: 2161–8.

17. Johnson JR, Delavari P, O'Bryan TT *et al.* Contamination of retail foods, particularly Turkey, from community markets (Minnesota, 1999–2000) with antimicrobial-resistant and extraintestinal pathogenic *Escherichia coli. Foodborne Pathog Dis* 2005; **2**: 38–49.

18. Carter MW, Oakton KJ, Warner M *et al.* Detection of extended-spectrum β-lactamases in Klebsiellae with the Oxoid combination disk method. *J Clin Microbiol* 2000; **38**: 4228–32.

19. Ensor VM, Livermore DM, Hawkey PM. A novel reverse-line hybridization assay for identifying genotypes of CTX-M-type extended-spectrum β-lactamases. *J Antimicrob Chemother* 2007; **59**: 387–95.

20. Vogel L, Jones G, Triep S *et al.* RAPD typing of *Klebsiella pneumoniae, Klebsiella oxytoca, Serratia marcescens* and *Pseudomonas aeruginosa* isolates using standardized reagents. *Clin Microbiol Infect* 1999; **5**: 270–6.

21. Chanawong A, M'Zali FH, Heritage J *et al.* Three cefotaximases, CTX-M-9, CTX-M-13, and CTX-M-14, among Enterobacteriaceae in the People's Republic of China. *Antimicrob Agents Chemother* 2002; **46**: 630–7.

22. Valverde A, Coque TM, Sanchez-Moreno MP *et al.* Dramatic increase in prevalence of fecal carriage of extended-spectrum β-lactamase-producing Enterobacteriaceae during nonoutbreak situations in Spain. *J Clin Microbiol* 2004; **42**: 4769–75.

23. Munday CJ, Whitehead GM, Todd NJ *et al.* Predominance and genetic diversity of community- and hospital-acquired CTX-M extended-spectrum β-lactamases in York, UK. *J Antimicrob Chemother* 2004; **54**: 628–33.

24. Woodford N, Fagan EJ, Ellington MJ. Multiplex PCR for rapid detection of genes encoding CTX-M extended spectrum β-lactamases. *J Antimicrob Chemother* 2006; **57**: 154–5.

25. Johnson TJ, Kariyawasam S, Wannemuehler Y *et al.* The genome sequence of avian pathogenic *Escherichia coli* strain O1:K1:H7 shares strong similarities with human extraintestinal pathogenic *E. coli* genomes. *J Bacteriol* 2007; **189**: 3228–36.

26. Johnson JR, Sannes MR, Croy C *et al.* Antimicrobial drug-resistant *Escherichia coli* from humans and poultry products, Minnesota and Wisconsin, 2002–2004. *Emerg Infect Dis* 2007; **13**: 838–46.

27. Warren RE, Harvey G, Carr R *et al.* Control of infection with extended-spectrum β-lactamase-producing organisms in hospital and the community. *Clin Microbiol Infect* 2008; **14** (Suppl 1): 124–33.

28. Doi Y, Adams J, O'Keefe A *et al.* Community-acquired extended-spectrum β-lactamase producers, United States. *Emerg Infect Dis* 2007; **13**: 1121–3.

Table 3. *E. coli* isolates with CTX-M ESBLs from chicken breast, as proportion of that cutting/packing station sampled

Cutting/packing station	Samples positive/samples taken
UK1	2/6
UK2	1/7
UK3	5/7
UK4	2/6
UK5	1/1
NE1	2/3

CTX-M-14 to be the second-most-prevalent UK genotype after CTX-M-15.

We hypothesized that chicken products imported into the UK potentially could act as a major source of gut colonization by avian strains of *E. coli* that carry $bla_{CTX\text{-}M}$ ESBL genes. Cooking does not necessarily prevent organisms from raw chicken, handled and cooked in a domestic setting, from colonizing the gut.[12] The phylogroups and serotypes of *E. coli* that cause urinary infection (uropathogenic or extraintestinal pathogenic *E. coli*) are restricted when compared with avian and human faecal isolates but are similar to avian pathogenic strains of *E. coli* (APEC).[14] The complete genome of an APEC strain was 95.5% identical to a human uropathogenic (UPEC) *E. coli* strain, and multi-locus sequence typing showed that some human UPEC strains were more similar to APEC than to other human UPEC strains.[25] Further, molecular comparisons of multiple virulence and antibiotic resistance factors suggest that human antimicrobial-resistant *E. coli* more closely resemble poultry strains than human antimicrobial-susceptible *E. coli*.[26] Early in the rise in CTX-M in the UK, data from a survey of faecal colonization showed a much wider range of both genotypes and host species than was then seen among in-patients.[23] Introduction of some strains with locally new CTX-M genotypes via imported food may lead to gut colonization that precedes urinary tract infection.[27] Differing current food and extraintestinal human genotypes may not preclude subsequent clinical infection with the food genotype. Gut colonization, when followed by urinary catheterization or personal hygiene problems, could explain the current epidemiology of ESBL producers, which occur particularly in the elderly in the UK in hospitals and the community. Ingested avian strains could transfer resistance or virulence factors to human pathogenic *E. coli*, although this would not explain multi-focal clonal spread. CTX-M-15 has now been described in retail chicken meat in the USA simultaneously with the first descriptions of community human cases with this genotype.[28]

Although *E. coli* strains with the dominant CTX-M-15 enzyme were not found in the current meat samples, we cannot discount the possibility that they have (or originally had) a source in foodstuffs. *E. coli* isolates with CTX-M-15 were recognized from 2001 in the UK, but first became widespread in 2003. A counter-explanation is that strains with CTX-M-15 were introduced by returning travellers or migrants from the Indian subcontinent, where the enzyme is extremely widespread[6] and from where it was first described. However, several of the early epicentres of clonal *E. coli* with CTX-M-15 enzymes (e.g. Ulster and Shrewsbury) do not have large migrant populations, arguing against spread mediated by human travel.

Given the frequent presence of *E. coli* strains with quinolone resistance and CTX-M genes in raw chicken breast on sale, sustained parallel surveillance of imported raw meat sources and human infection is necessary to establish whether there is a related, gradual change in the prevalent CTX-M types and whether resistance genes and plasmids in isolates from raw food are the progenitors of changes in the epidemiology of CTX-M enzymes in clonal isolates. Packaging information should indicate the country where chicken is reared, and the bacteriological standards for raw poultry meat should be reviewed. The high prevalence of multiresistant ESBL-producing *E. coli* in imported chicken is undesirable. Although molecular epidemiology did not show that raw poultry meat is an ongoing source for the current clinical infections in the UK, this meat has the potential to act as a source for faecal colonization with ESBL-producing *E. coli* as a prelude to extraintestinal infection.

Acknowledgements

These data were presented as a poster at the European Congress of Clinical Microbiology and Infectious Diseases, Munich, 2007. We thank the local authority environmental health officers of the Shropshire and West Midlands Food Liaison Groups for collection of the samples and enquiries on the countries of origin.

Funding

V. M. E. was supported in part for undertaking the molecular work by a grant from the British Society for Antimicrobial Chemotherapy awarded to P. M. H. Analytical work on food samples was supported by internal funding from the Health Protection Agency for the normal food, water and environmental activity of its laboratories and Collaborating Laboratories.

Transparency declarations

None to declare.

Contributions to the study: R. E. W., hypothesis, study design and manuscript preparation; P. M. H. and K. N., study design and manuscript preparation; V. M. E., molecular strain determination, data analysis and RAPD; P. O'N., liaison with food liaison groups; M. H., V. B. and J. T., culture and other bacteriology of food samples; D. M. L. and N. W., preliminary molecular analysis and manuscript preparation.

References

1. Woodford N, Ward ME, Kaufmann ME *et al.* Community and hospital spread of *Escherichia coli* producing CTX-M extended-spectrum β-lactamases in the UK. *J Antimicrob Chemother* 2004; **54**: 735–43.

Table 1. *E. coli* isolates with CTX-M ESBLs from chicken breast, by country of origin

Origin	Total positive/ total tested	CTX-M gene present			
		CTX-M-1	CTX-M-2	CTX-M-8	CTX-M-14
UK	1/62	1	0	0	0
Ireland	0/3	0	0	0	0
Brazil	4/10	0	4	0	0
Brazil/Poland/France[a]	3/4	0	3	0	0
Poland	0/4	0	0	0	0
The Netherlands	2/2	0	2	0	0
Spain, France, Denmark and Germany[b]	0/4	0	0	0	0
Unknown[c]	7/40	0	1	1	5[d]
Total	17/129	1	10	1	5

[a]Precise country of rearing not stated on packaging.
[b]Only single samples from each country available.
[c]Country of rearing not identified on packaging.
[d]All chicken meat containing *E. coli* with CTX-M-14 enzymes was purchased from two major supermarket chains and was processed at a minimum of two UK cutting/packing stations.

Table 2. *E. coli* isolates with CTX-M ESBLs from chicken breast, by sample source

Sample	Retailer	Outlet town/city	Packing station[b]	Lot	Origin	Enzyme
1	A	1	unknown	unknown	unknown	CTX-M-14
2	A	2	unknown	unknown	unknown	CTX-M-14
3	A	1	UK1	A	unknown	CTX-M-14
4[a]	A	1	UK1	A	unknown	CTX-M-14
5	B	1	unknown	unknown	unknown	CTX-M-8
6	C	3	UK2	B	not known	CTX-M-14
7	D	4	UK3	C	Brazil/Poland/France	CTX-M-2
8	D	4	UK3/BR1	D	Brazil	CTX-M-2
9	D	5	UK3	E	Brazil	CTX-M-2
10	D	6	UK3	F	Brazil/Poland/France	CTX-M-2
11	D	7	UK3	H	Brazil/Poland/France	CTX-M-2
12	E	8	UK4/BR2	G	Brazil	CTX-M-2
13[a]	E	8	UK4/BR2	G	Brazil	CTX-M-2
14	F	9	UK5	I	UK	CTX-M-1
15	G	4	unknown	unknown	unknown	CTX-M-2
16	H	1	NE1	H	Holland	CTX-M-2
17[a]	H	1	NE1	H	Holland	CTX-M-2

[a]Sample from same batch as sample above.
[b]Cutting/packing stations are designated as located in the UK, Brazil (BR) or Netherlands (NE) followed by a sequential number.

Midlands. This low UK rate may reflect restrained use of antibiotics such as ceftiofur and enrofloxacin in the UK poultry production.

Sixteen more isolates with CTX-M enzymes were found, however, in isolates from imported raw chicken. In particular, 40% of the imported Brazilian chicken and three of four samples with an aggregated origin of 'Brazil/Poland/France' contained *E. coli* producing CTX-M-2 enzyme, as did two chicken samples from the same lot from the Netherlands. The latter finding correlates with a recent Dutch report of $bla_{CTX\text{-}M\text{-}2}$ in *S. enterica* Virchow from broiler faeces.[8] CTX-M-2 is rare in human clinical isolates in Europe but well known to be the prevalent CTX-M type in clinical isolates from Argentina, so its isolation from Brazil-reared chicken is unsurprising. The second most frequently encountered genotype was CTX-M-14 from samples packed for two supermarket chains and handled in at least two UK packing/cutting stations but where the country of rearing was not recorded. Originally described in far Eastern countries,[21] this type has spread and, together with the related CTX-M-9 type, is now prevalent also in Spain.[22] Moreover, both a survey of human faecal carriage in 2003 in York[23] and a recent survey[24] of strains from human infections found

clinical O25 isolates in Shropshire and most of the isolates nationally are also quinolone-resistant by a mechanism independent of ESBL production.[1–3] Similar dramatic increases in ESBL-producing *E. coli* have occurred in many other countries, often associated with community acquisition and the problem has been described as a pandemic.[3,4] Plasmid-mediated CTX-M β-lactamase genes originated by mobilization from the genus *Kluyvera* and subsequent mutation have resulted in the emergence of nearly 50 distinct variants.[4] Several of these have a distinct geographical distribution worldwide, e.g. CTX-M2 in South America, Israel and Japan, CTX-M-14 in China, and CTX-M-9/14 in Spain.[5] This probably arises from the transfer of bla_{CTX-M} genes from *Kluyvera* into *E. coli* and subsequent accumulation of mutations locally followed by widespread distribution at these locations. CTX-M-15 enzyme occurs worldwide but is the only genotype present in India, which has been suggested as its origin.[6] The recognition of genotypes that are rare in the UK but very common in other parts of the world suggests direct/indirect importation, although it could sometimes represent a recurrence of the same mutation.

Food is an important vehicle for antibiotic-resistant gastrointestinal pathogens such as *Campylobacter jejuni* and *Salmonella enterica*. *S. enterica* with CTX-M enzymes are increasingly reported from food animals, particularly poultry, and the genotypes sometimes correspond with the locally dominant human types,[7] although this is not always the case.[8] Likewise, *E. coli* strains in food animals in Japan,[9] Spain[10] and Hong Kong[11] tend to carry the same CTX-M enzyme variants locally dominant in human isolates, but food is not confirmed as a human source. It is widely argued that as meat products are cooked, there is little likelihood that antibiotic-resistant bacteria present in the raw material will colonize the human gut. This view is challenged by the work from 30 years ago, which clearly demonstrated the colonization of humans by antibiotic-resistant *E. coli* in the course of preparing and eating cooked chicken in the home.[12] Moreover, there is a substantial overlap between the phylogroups, serotypes and virulence factors of *E. coli* from human urinary infections and those of poultry strains of *E. coli* associated with the disease of avian colibacillosis.[13,14] *E. coli* serotype O25 has been isolated from chickens in India,[15] where CTX-M-15 was originally described. Raw retail poultry in the USA is frequently reported to contain quinolone-resistant *E. coli* with human urinary infection virulence factors.[16,17] On the basis of these earlier findings, we examined chicken breasts as a potential reservoir of quinolone-resistant ESBL-producing *E. coli* and tested the hypothesis that the rapid multi-focal proliferation of *E. coli* with CTX-M-15 β-lactamase might be related to consumption of chicken breast meat currently on sale in the UK.

Methods

During January/February and July/August 2006, 129 fresh and frozen raw chicken breast fillets (originating from the UK and other countries) were purchased by local authority environmental health officers from 18 different retail outlets, including 13 major supermarket chains in Shropshire and Birmingham, UK. Sampling was unstructured and convenience-based. Sampling depended on the number of products available on the day and sought to represent both imported and UK products. Twenty-five grams of the chicken meat was macerated with 225 mL of buffered peptone water (Oxoid, Basingstoke, Hants, UK) and incubated for 18 h at 37°C. Ten microlitre aliquots of the broth cultures were then plated onto CLED agar (Oxoid) containing 8 mg/L ciprofloxacin, and a cefpodoxime disc (10 μg) was placed on the agar surface. This recovery method was insensitive as it did not involve selective liquid enrichment and was primarily selective for quinolone-resistant organisms; it would not have grown any that had ESBLs but which remained fluoroquinolone susceptible, although these are uncommon in human isolates in the UK. After incubation, resistant colonies from within the cefpodoxime zone were retained, initially confirmed as *E. coli* using chromogenic urinary agar (BBL, Oxford, UK), subsequently confirmed as *E. coli* using API20E identification, and were investigated for ESBL production using the Oxoid combination disc test.[18] Isolates found to be ESBL-positive were screened for bla_{CTX-M} by multiplex PCR,[14] and reverse-line hybridization was used to identify the specific bla_{CTX-M} genotypes,[19] which were then confirmed by sequencing. The clonality of bla_{CTX-M}-bearing isolates was investigated using RAPD genomic fingerprinting.[20]

Results

Of the 62 packaged raw breasts from chicken reared in the UK, only one yielded a quinolone-resistant *E. coli* with a bla_{CTX-M} gene, compared with 9 of 27 of those identifiable as reared overseas and 7 of 40 for which the country of rearing was not stated. The UK-reared sample carried *E. coli* with a CTX-M-1 enzyme. No isolates with CTX-M-15 enzyme were found. However, isolates with CTX-M-2 and CTX-M-14 enzymes were common and single strains producing CTX-M-1 or CTX-M-8 enzymes were recovered (Table 1). Isolates with CTX-M-2 genes were recovered in imports from Brazil (4/10 samples) and 3/4 samples of pooled chicken meat from France, Poland and Brazil, as well as the Netherlands. Isolates with CTX-M-14 and M-8 enzymes were recovered from meat where the country in which the chicken was reared was not indicated on the packaging. Overall, the chicken packaging contained references to 27 cutting/packing stations, but not all indicated the country in which the chicken was reared. Enquiry was made of UK cutting/packing stations to determine the origin from product code numbers, but this information was not always supplied. Coded details of supermarket of origin, retail outlet, cutting/packing station and lot, for all samples yielding positive isolates are given in Table 2. The proportions of samples yielding positive isolates from cutting/packing stations yielding any positives are given in Table 3. Positive results were not related to the presence of skin on the breasts (data not shown). RAPD typing showed that all the bla_{CTX-M}-positive isolates were unique.

Discussion

In contrast to the recent papers from Japan, Hong Kong and Spain reporting poultry carriage of the same CTX-M genotypes of *E. coli* in food as are locally dominant in human infections,[9–11] we did not find a single strain of *E. coli* with the clinically predominant $bla_{CTX-M-15}$ genotype.

British poultry yielded only a single strain with a CTX-M enzyme, specifically CTX-M-1 enzyme, which has only recently been reported causing infection in the UK in the West

Journal of Antimicrobial Chemotherapy (2008) **61**, 504–508
doi:10.1093/jac/dkm517
Advance Access publication 25 January 2008

JAC

Imported chicken meat as a potential source of quinolone-resistant *Escherichia coli* producing extended-spectrum β-lactamases in the UK

R. E. Warren[1]*, V. M. Ensor[2], P. O'Neill[1], V. Butler[1], J. Taylor[1], K. Nye[3], M. Harvey[3], D. M. Livermore[4], N. Woodford[4] and P. M. Hawkey[2,3]

[1]*Microbiology Laboratory, Shrewsbury and Telford Hospital NHS Trust, Shrewsbury SY3 8XQ, UK;* [2]*Division of Immunity and Infection, The Medical School, University of Birmingham, Birmingham B15 2TT, UK;* [3]*West Midlands Health Protection Agency, Heart of England NHS Foundation Trust, Birmingham B9 5SS, UK;* [4]*Antibiotic Resistance and Monitoring Reference Laboratory, Health Protection Agency, 61 Colindale Avenue, London NW9 5EQ, UK*

Received 23 September 2007; returned 4 October 2007; revised 4 December 2007; accepted 5 December 2007

***Objectives*: *Escherichia coli* producing CTX-M-15 enzyme began to rapidly spread in the UK from around 2003 but other types also occur, notably CTX-M-14. We examined breasts from UK-reared (n = 62) and imported (n = 27) chickens as potential sources of quinolone-resistant *E. coli* with bla_{CTX-M} genes. A further 40 samples for which the country of rearing could not be identified were examined.**

***Methods*: During 2006, 129 fresh and frozen chicken breast fillets were purchased from retail outlets in the West Midlands. These were cultured for *E. coli* on CLED agar containing 8 mg/L ciprofloxacin and carrying a 10 μg cefpodoxime disc. Resistant isolates were identified and typed by RAPD fingerprinting; bla_{CTX-M} was identified by PCR and genotyped by reverse-line hybridization.**

***Results*: The country of rearing was identified from the packaging for 89 of 129 purchased samples. Only one of the 62 UK-reared chicken samples carried *E. coli* producing a CTX-M-1 enzyme, whereas 10 of 27 samples reared overseas had *E. coli* with CTX-M enzymes. Specifically, 4/10 Brazilian, 3/4 Brazilian/Polish/French, and 2/2 Dutch samples had *E. coli* with CTX-M-2 enzymes. Six of 40 samples for which the country of rearing was not known had producers of CTX-M enzymes, 5 of them with CTX-M-14.**

***Conclusions*: Quinolone-resistant *E. coli* with various CTX-M β-lactamase genes that are common in human infections worldwide were found in imported chicken breasts, indicating a possible source for gut colonization. Samples from Brazil were commonly positive for *E. coli* with CTX-M-2, the dominant bla_{CTX-M} genotype from human infections in South America, which is currently rare in clinical infections in the UK. CTX-M-15, the dominant CTX-M type in human infections in the UK, was not found in chicken isolates, suggesting that the UK-reared chickens are not a reservoir of CTX-M-15.**

Keywords: ESBLs, food, quinolones, Enterobacteriaceae

Introduction

Extended-spectrum β-lactamases (ESBLs) are bacterial enzymes that degrade oxyimino-cephalosporins such as cefotaxime and ceftazidime. They are spread among bacterial species by plasmids, often carrying multiple antibiotic resistance genes. Since 2003, multiply-resistant *Escherichia coli* strains producing the CTX-M-15 type ESBL have become widespread as agents of urinary and other infections in many primary and secondary care centres in the UK. Five closely related clones, all of serotype O25 and phylogroup B2, are common, along with many clonally diverse producers. The increase in CTX-M-15-producing *E. coli* in the UK in 2003, with the simultaneous multicentric appearance of clonally related strains, is unexplained. A nationally distributed food source cannot be excluded, due to the lack of sampling at the time of onset of the UK outbreak. All the

*Corresponding author. Tel: +44-1743-261163; Fax: +44-1743-261165; E-mail: roderic.warren@homecall.co.uk

24. Murray AC, Kuskowski MA, Johnson JR. Virulence factors predict *Escherichia coli* colonization patterns among human and animal household members. Ann Intern Med. 2004;140:848–9.
25. Manges AR, Johnson JR, Riley LW. Intestinal population dynamics of urinary tract infection-causing *Escherichia coli* within heterosexual couples. Curr Issues Intest Microbiol. 2004;5:49–57.
26. Skyberg JA, Johnson TJ, Johnson JR, Clabots C, Logue CM, Nolan LK. Acquisition of avian pathogenic *Escherichia coli* plasmids by a commensal *E. coli* isolate enhances its abilities to kill chicken embryos, grow in human urine, and colonize the murine kidney. Infect Immun. 2006;74:6287–92.
27. Johnson JR, Clermont O, Menard M, Kuskowski MA, Picard B, Denamur E. Experimental mouse lethality of *Escherichia coli* isolates in relation to accessory traits, phylogenetic group, and clinical source. J Infect Dis. 2006;194:1141–50.
28. Winokur PL, Vonstein DL, Hoffman EK, Uhlenhopp EK, Doern GV. Evidence for transfer of CMY-2 AmpC β-lactamase plasmids between *Escherichia coli* and *Salmonella* isolates from food animals and humans. Antimicrob Agents Chemother. 2001;45:2716–22.
29. Johnson JR, Stell A, Delavari P. Canine feces as a reservoir of extraintestinal pathogenic *Escherichia coli.* Infect Immun. 2001;69:1306–14.
30. Tartof SY, Solberg OD, Manges AR, Riley LW. Analysis of a uropathogenic *Escherichia coli* clonal group by multilocus sequence typing. J Clin Microbiol. 2005;43:5860–4.

Address for correspondence: James R. Johnson, Infectious Diseases (111F), Minneapolis Veterans Affairs Medical Center, 1 Veterans Dr, Minneapolis, MN 55417, USA; email: johns007@umn.edu

and resistance elements (*28*). Use of multiple comparisons increased the likelihood of spurious associations (which we addressed by specifying a strict criterion for statistical significance), whereas the small sample size in certain subgroups reduced power for finding true associations.

Strengths of the study include substantial overall sample size, standardized concurrent processing of fecal and poultry samples, close matching of human and poultry samples, extensive molecular typing using virulence-relevant markers, and use of multiple analytical modalities. Additionally, we examined clinically relevant resistance phenotypes.

In summary, our findings suggest that in a contemporary US-based population, many human-source drug-resistant fecal *E. coli* isolates more likely originated in poultry than in humans, whereas drug-resistant poultry isolates likely derive from drug-susceptible poultry isolates. Our data extend this paradigm to clinically relevant agents other than fluoroquinolones, heighten concerns regarding the potential human health risk for antimicrobial drug use in poultry production, and suggest that avoidance of poultry consumption may not reliably provide personal protection.

Acknowledgments

We thank Mary Vandermause, Burney Kieke, and Amy Kieke for assistance with the study design, logistics, and data management.

This study was supported by the Office of Research and Development, Medical Research Service, Department of Veterans Affairs; Centers for Disease Control and Prevention grants R01-CI000204 (to J.R.J.) and RS1-CCR520634 (to E.A.B.); the University of Minnesota Rapid Agricultural Response Fund (to J.B.); and the Minnesota Department of Health Emerging Infections program.

Dr Johnson is professor of medicine and director of the Infectious Diseases Fellowship Program at the University of Minnesota and an infectious diseases physician and director of the Molecular Epidemiology Laboratory at the Minneapolis Veterans Affairs Medical Center. His research interests include virulence mechanisms, molecular epidemiology, antimicrobial drug resistance, evolution, reservoirs, and transmission pathways of extraintestinal pathogenic *E. coli*.

References

1. Gupta K, Hooton TM, Stamm WE. Increasing antimicrobial resistance and the management of uncomplicated community-acquired urinary tract infections. Ann Intern Med. 2001;135:41–50.
2. Pitout JD, Nordmann P, Laupland KB, Poirel L. Emergence of *Enterobacteriaciae* producing extended spectrum β-lactamases (ESBLs) in the community. J Antimicrob Chemother. 2005;56: 52–9.
3. Garau J, Xercavins M, Rodriguez-Carballeira M, Gomez-Vera JR, Coll I, Vidal D, et al. Emergence and dissemination of quinolone-resistant *Escherichia coli* in the community. Antimicrob Agents Chemother. 1999;43:2736–41.
4. Russo TA, Johnson JR. Medical and economic impact of extraintestinal infections due to *Escherichia coli*: an overlooked epidemic. Microbes Infect. 2003;5:449–56.
5. Linton AH. Animal to man transmission of *Enterobacteriaceae*. R Soc Health J. 1977;97:115–8.
6. Jones TF, Schaffner W. New perspectives on the persistent scourge of foodborne disease. J Infect Dis. 2005;191:1029–31.
7. Collignon P, Angulo FJ. Fluoroquinolone-resistant *Escherichia coli*: food for thought. J Infect Dis. 2006;194:8–10.
8. Johnson JR, Murray AC, Gajewski A, Sullivan M, Snippes P, Kuskowski MA, et al. Isolation and molecular characterization of nalidixic acid-resistant extraintestinal pathogenic *Escherichia coli* from retail chicken products. Antimicrob Agents Chemother. 2003;47:2161–8.
9. Johnson JR, Delavari P, O'Bryan TT, Smith K, Tatini S. Contamination of retail foods, particularly turkey, from community markets (Minnesota, 1999–2000) with antimicrobial-resistant and extraintestinal pathogenic *Escherichia coli*. Foodborne Pathog Dis. 2005;2:38–49.
10. Johnson JR, Kuskowski MA, Smith K, O'Bryan TT, Tatini S. Antimicrobial-resistant and extraintestinal pathogenic *Escherichia coli* in retail foods. J Infect Dis. 2005;191:1040–9.
11. Schroeder CM, White DG, Ge B, Zhang Y, McDermott PF, Avers S, et al. Isolation of antimicrobial-resistant *Escherichia coli* from retail meats purchased in Greater Washington, DC, USA. Int J Food Microbiol. 2003;85:197–202.
12. Johnson JR, Kuskowski MA, Menard M, Gajewski A, Xercavins M, Garau J. Similarity of human and chicken-source *Escherichia coli* isolates in relation to ciprofloxacin resistance status. J Infect Dis. 2006;194:71–8.
13. Mellon M, Benbrook C, Lutz Benbrook K. Hogging it. Estimates of antimicrobial abuse in livestock. Cambridge (MA): UCS Publications; 2001.
14. Kieke AL, Borchardt MA, Kieke BA, Spencer SK, Vandermause MF, Smith KE, et al. Use of streptogramin growth promoters in poultry and isolation of streptogramin-resistant *Enterococcus faecium* from humans. J Infect Dis. 2006;194:1200–8.
15. Johnson JR, Murray AC, Kuskowski MA, Schubert S, Prere MF, Picard B, et al. Distribution and characteristics of *Escherichia coli* clonal group A. Emerg Infect Dis. 2005;11:141–5.
16. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl Environ Microbiol. 2000;66:4555–8.
17. Johnson JR, Stell AL. Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. J Infect Dis. 2000;181:261–72.
18. Gower JC. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika. 1966;53:325–38.
19. Peakall R, Smouse PE. GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes. 2006;6:288–95.
20. Sokal RR, Sneath PH. Construction of a taxonomic system. In: Sokal RR, Sneath PHA, editors. Principles of numerical taxonomy. San Francisco: W.H. Freeman; 1963.
21. Gorbach SL. Antimicrobial use in animal feed--time to stop. N Engl J Med. 2001;345:1202–3.
22. Singer RS, Finch R, Wegener HC, Bywater R, Walters J, Lipsitch M. Antibiotic resistance–the interplay between antibiotic use in animals and human beings. Lancet Infect Dis. 2003;3:47–51.
23. Hannah EL, Angulo FJ, Johnson JR, Haddadin B, Williamson J, Samore MH. Drug-resistant *Escherichia coli*, rural Idaho. Emerg Infect Dis. 2005;11:1614–7.

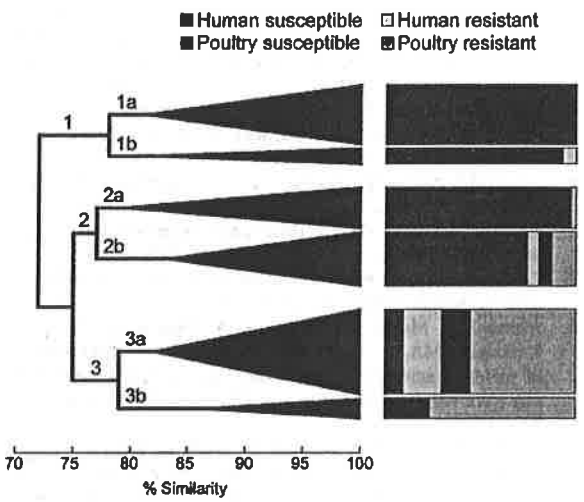


Figure 3. Dendrogram based on extended virulence profiles of 243 extraintestinal pathogenic *Escherichia coli* isolates from human feces and poultry products, Minnesota and Wisconsin, 2002–2004. The dendrogram (shown here in simplified form) was constructed by using the unweighted pair group method with arithmetic averages based on pairwise similarity relationships according to the aggregate presence or absence of 60 individual virulence genes plus phylogenetic group (A, B1, B2, D). Triangles indicate arborizing subclusters. Major clusters 1, 2, and 3, and subclusters 1a, 1b, 2a, 2b, 3a, and 3b are indicated. Colored boxes to right of dendrogram show the distribution (by source group) of constituent members of each subcluster. Resistant, resistant to trimethoprim-sulfamethoxazole, nalidixic acid (quinolones), and ceftriaxone or ceftazidime (extended-spectrum cephalosporins). Susceptible, susceptible to all these agents.

susceptible, poultry-source *E. coli* by conversion to resistance. This most plausibly would occur within the avian fecal flora under selection pressure from on-farm use of antimicrobial drugs.

Our findings closely resemble those of a recent study of ciprofloxacin-resistant *E. coli* from humans and chickens in the late 1990s in Barcelona, Spain (*12*). These data indicate that these relationships remain valid and are applicable in the United States, to additional resistance phenotypes (specifically quinolones, TMP-SMZ, and extended-spectrum cephalosporins), and to retail poultry products (*12*). Moreover, similar results were obtained with retail poultry products and poultry carcasses from processing plants. This implies that drug-resistant poultry-source *E. coli* isolates originate in the birds, rather than being introduced from some exogenous reservoir later during the packaging and distribution process. This in turn suggests that on-farm practices, including use of antimicrobial agents for growth promotion, metaphylaxis, and therapy (*21*,*22*), may influence characteristics of *E. coli* that contaminate retail poultry products and, seemingly, are then transmitted to humans (*7*).

The greater overall similarity of drug-resistant human isolates to poultry isolates than to drug-susceptible human isolates applied not only to the hospital patient isolates compared with isolates from conventionally raised poultry, but also to the isolates from vegetarians compared with isolates from poultry raised with no antibiotics. This was surprising because the vegetarians ostensibly did not consume poultry and, therefore, should not have been directly exposed to poultry-source *E. coli*. However, this seeming paradox is consistent with the difficulty in confirming poultry consumption (along with most other individual-level exposures) as an epidemiologic risk factor for colonization with drug-resistant *E. coli* isolates among community-dwelling persons ([*23*]; J.R. Johnson, unpub. data). Assuming that the drug-resistant human isolates were derived from poultry, occurrence of poultry-source *E. coli* in both vegetarians and persons with conventional diets suggests that poultry-source drug-resistant *E. coli* may spread extensively through the human population without requiring individual exposure to poultry products. This suggestion would be consistent with evidence that household-level risk factors may be more predictive of colonization with drug-resistant *E. coli* than individual-level risk factors, and that household members often share *E. coli* clones with each another (*23*–*25*). The mechanisms for such diffusion, and methods to block the entry of such strains into the human population and their subsequent spread, need to be defined.

The virulence potential for humans of the present drug-resistant human and poultry *E. coli* isolates, which is related to their direct threat to human health, is unknown. Predictions regarding virulence potential await molecular comparisons with human clinical isolates (*9*,*10*,*12*) and experimental virulence assessment in vivo (*26*,*27*). Nonetheless, the abundance of ExPEC-associated virulence genes in some of these strains is of concern because it suggests a high likelihood of virulence. This would augment any health threat these strains may pose as passive vehicles for drug-resistance genes (*6*,*7*).

Potential limitations of this study warrant comment. Because we did not examine alternative sources for drug-resistant human isolates, we cannot exclude the possibility that other foods (*28*) or nonfood reservoirs (*29*) might yield even closer similarities to drug-resistant human isolates. Whether persons in the study consumed poultry products from the same lots or suppliers as those sampled is not known. Because the study was conducted in Minnesota and Wisconsin in mostly rural communities and with newly hospitalized patients and nonhospitalized vegetarians, generalizability of the results is unknown. We combined several resistance phenotypes because of low frequencies, which may have obscured differences. We also did not assess other molecular characteristics of strains, e.g., pulsed-field gel electrophoresis profiles (*12*), sequence types (*30*),

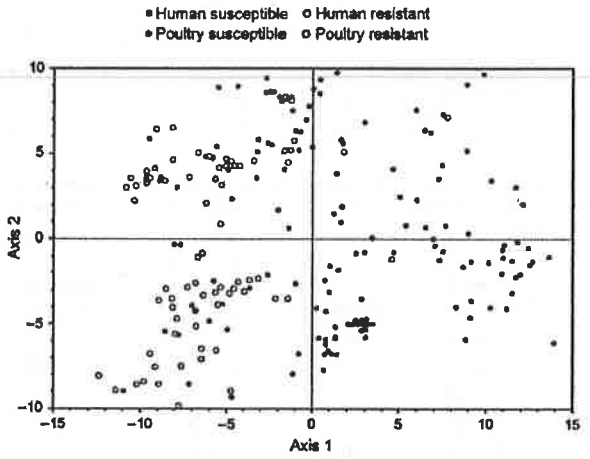


Figure 1. Principal coordinates analysis of distribution of 243 extraintestinal pathogenic *Escherichia coli* isolates from human feces and poultry products, Minnesota and Wisconsin, 2002–2004, on the axis 1–axis 2 plane. Data include extended virulence genotypes (60 traits) and phylogenetic group (A, B1, B2, D). The axes have no units; they reflect the total score for each isolate derived by summing the isolate's partial score for each variable, which is the product of the loading score assigned to the particular variable for a given axis and the isolate's status for that variable. Axis 1 (positive values to right, negative values to left of central vertical line) accounted for 37% of total variance and showed significant differences between susceptible human isolates versus each of the other groups. Axis 2 (positive values above, negative values below central horizontal line) accounted for 20% of total variance and did not show any significant between-group differences. Resistant, resistant to trimethoprim-sulfamethoxazole, nalidixic acid (quinolones), and ceftriaxone or ceftazidime (extended-spectrum cephalosporins). Susceptible, susceptible to all these agents (regardless of other possible drug resistance).

Dendrogram of Extended Virulence Profiles and Phylogenetic Group

Phylogenetic group and extended virulence profiles among the 243 available ExPEC isolates also were used to construct a similarity dendrogram. The dendrogram showed 3 major clusters, each of which contained 2 prominent subclusters (Figure 3). Isolates were distributed by cluster and subcluster according to source and resistance group; that is, drug-susceptible human isolates accounted for almost all of subclusters 1a, 1b, and 2a. In contrast, drug-resistant human isolates were confined largely to subcluster 3a. Poultry isolates, whether resistant or susceptible, were confined almost entirely to subclusters 2b, 3a, and 3b. Thus, compared with drug-susceptible human isolates, drug-resistant human isolates were significantly more likely to occur within a subcluster, or major cluster, that also contained poultry isolates ($p<0.001$ for each comparison).

The possible effects of nonindependence among multiple isolates acquired from the same sample were assessed by limiting the analysis to a single isolate per sample, keeping a drug-susceptible isolate (if available) and randomly selecting among multiple drug-resistant isolates where required. This resulted in reduced sample sizes of 681 (total population) and 226 (ExPEC population). The analysis results closely mirrored the pattern of significant findings obtained in the full samples.

Discussion

In this study, we analyzed the phylogenetic distribution and virulence genotypes of drug-susceptible and drug-resistant *E. coli* isolates from human volunteers and poultry products in Minnesota and Wisconsin. We found that drug-resistant human isolates, although overlapping somewhat with drug-susceptible human isolates, were more similar overall to poultry isolates than to drug-susceptible human isolates. In contrast, drug-susceptible human isolates differed from poultry isolates. This relationship was observed consistently with diverse analytical approaches and various stratifications of the population. It suggests that many of the drug-resistant human isolates were more likely to have originated in poultry (or a similar nonhuman reservoir) and to have been acquired by humans when these isolates were already drug resistant, than to have emerged de novo in humans by conversion of drug-susceptible human isolates to drug-resistant isolates.

We also found that, regardless of analytical approach and population analyzed, resistant and susceptible poultry isolates were highly similar. This suggests that the resistant poultry isolates likely derived from antimicrobial drug–

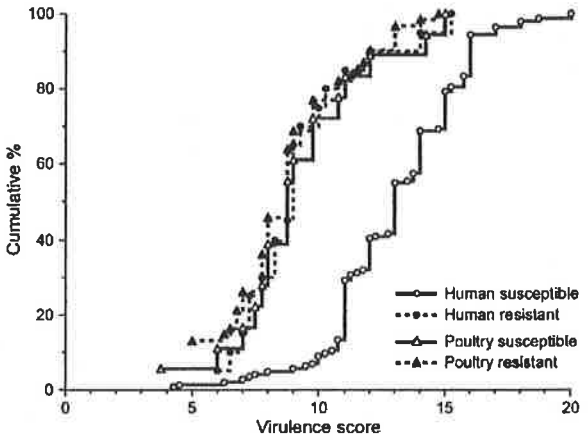


Figure 2. Distribution of virulence factor scores by source and resistance status among 243 extraintestinal pathogenic *Escherichia coli* isolates from human feces and poultry products, Minnesota and Wisconsin, 2002–2004. Resistant, resistant to trimethoprim-sulfamethoxazole, nalidixic acid (quinolones), and ceftriaxone or ceftazidime (extended-spectrum cephalosporins). Susceptible, susceptible to all these agents (regardless of other possible resistances). The virulence scores of the susceptible human isolates are an average of ≈4 points greater than those of the resistant human isolates or poultry isolates.

analysis. Drug-susceptible human isolates had the highest scores (median 13.0, range 4.25–20.0). Drug-resistant human and poultry isolates had significantly lower scores that did not differ between humans and poultry (median 9.0, range 6.0–15.25, and median 8.75, range 3.75–15.0, respectively; vs. drug-susceptible human isolates, p<0.001). Similar results were obtained when isolates from hospital patient fecal samples were compared separately with the conventionally raised poultry isolates or when isolates from vegetarian fecal samples were compared separately with isolates from poultry raised without antibiotics (data not shown).

Table 2. Bacterial traits by source and antimicrobial drug resistance in 243 extraintestinal pathogenic *Escherichia coli* (ExPEC) isolates from human feces and poultry products, Minnesota and Wisconsin, 2002–2004*

	Prevalence, no. (%)				p value¶		
Trait†‡§	Total (n = 243)	Human, susceptible (n = 144)	Human, resistant (n = 20)	Poultry (n = 79)	HS vs. HR	HS vs. all poultry	HR vs. all poultry
Group A	20 (8)	5 (3)	5 (25)	10 (13)	≤0.01#		
Group B1	7 (3)	0	0	7 (9)		≤0.001#	≤0.001#
Group B2	154 (63)	125 (87)	6 (30)	23 (29)		≤0.001	
Group D	62 (26)	14 (10)	9 (45)	39 (49)		≤0.001#	
papA	117 (48)	97 (67)	7 (35)	13 (16)	≤0.01	≤0.001	
F10 allele	38 (16)	32 (10)	5 (25)	1 (1)		≤0.001	≤0.001
F16 allele	12 (5)	5 (3)	5 (25)	2 (3)	≤0.01#		≤0.01
F48 allele	21 (9)	21 (15)	0	0		≤0.001	
papG III	44 (18)	44 (31)	0	0	≤0.01	≤0.001	
sfa/focDE	62 (26)	61 (42)	1 (5)	0	≤0.001	≤0.001	
sfaS	35 (14)	33 (23)	1 (5)	1 (1)		≤0.001	
focG	13 (5)	12 (8)	1 (5)	0		≤0.01	
afa/draBC	15 (6)	11 (8)	4 (20)	0		≤0.01	≤0.001
iha	52 (22)	38 (26)	16 (80)	0	≤0.001#	≤0.001	≤0.001
hra	108 (44)	67 (47)	2 (10)	39 (49)	≤0.001		≤0.01#
cnf1	54 (22)	51 (35)	2 (10)	1 (1)		≤0.001	
hlyD	67 (28)	67 (28)	2 (10)	2 (3)	≤0.01	≤0.001	
hlyF	73 (30)	28 (19)	1 (5)	44 (57)		≤0.001#	≤0.001#
sat	61 (25)	46 (32)	15 (75)	0 (0)	≤0.001#	≤0.001#	≤0.001#
pic	34 (14)	30 (21)	0	4 (5)		≤0.01	
vat	131 (54)	113 (78)	3 (15)	15 (19)	≤0.001	≤0.001	
astA	48 (20)	7 (5)	1 (5)	40 (51)		≤0.001#	≤0.001#
iutA	162 (67)	67 (47)	18 (90)	77 (97)		≤0.001#	
iroN	118 (49)	78 (54)	3 (15)	37 (47)	≤0.001		≤0.01#
fyuA	199 (82)	138 (96)	17 (85)	44 (56)		≤0.001	
kpsM II	215 (89)	137 (95)	16 (80)	62 (78)		≤0.001	
K5 *kpsM*	35 (14)	28 (19)	4 (20)	3 (4)		≤0.001	
iss	69 (28)	23 (16)	2 (10)	44 (56)		≤0.001#	≤0.001#
usp	144 (59)	127 (88)	6 (30)	11 (14)	≤0.001	≤0.001	
H7 *fliC*	52 (21)	52 (36)	0	0	≤0.001	≤0.001	
ompT	184 (76)	131 (91)	9 (50)	40 (51)	≤0.01	≤0.001	
malX	152 (63)	134 (93)	7 (35)	1 (14)	≤0.001	≤0.001	

*Susceptible, susceptible to trimethoprim-sulfamethoxazole, nalidixic acid (quinolones), and ceftriaxone or ceftazidime (extended-spectrum cephalosporins), regardless of other possible drug resistance; resistant, resistant to ≥1 of the following: trimethoprim-sulfamethoxazole, nalidixic acid (quinolones), and ceftriaxone or ceftazidime (extended-spectrum cephalosporins).
†Traits are shown that showed p≤0.01 for ≥1 comparison each. Groups A, B1, B2, and D, major *E. coli* phylogenetic groups; *papA*, P fimbriae structural subunit with variants F10, F16, and F48; *papG* III, variant P adhesin; *sfa/focDE*, S and F1C fimbriae; *sfaS*, S fimbriae; *focG*, F1C fimbriae; *afa/draBC*, Dr binding adhesins; *iha*, adhesin-siderophore receptor; *hra*, pathogenicity island marker; *cnf1*, cytotoxic necrotizing factor 1; *hlyD*, α-hemolysin; *hlyF*, variant hemolysin; *sat*, secreted autotransporter toxin; *pic*, autotransporter protease; *vat*, vacuolating autotransporter; *astA*, enteroaggregative *E. coli* toxin; *iutA*, aerobactin system; *iroN*, siderophore receptor; *fyuA*, yersiniabactin receptor; *kpsM* II, group 2 capsule; K5 *kpsM*, *kpsM* II variant; *iss*, increased serum survival; *usp*, uropathogenic-specific protein; H7 *fliC*, flagellar variant; *ompT*, outer membrane protease; *malX*, pathogenicity island marker.
‡Traits that did not show p<0.01 but were detected in ≥1 isolate each include the F7–2, F8, F9, F11, F12, F12, F14, and F15 *papA* alleles, *papC* (P fimbriae assembly), *papEF* (P fimbriae tip pilins), *papG* alleles I and II (both internal and flanking sequences), *afaE8* (variant Dr binding adhesin), *gafD* (G fimbriae), F17 fimbriae, *fimH* (type 1 fimbriae), *clpG* (adhesin), *cdtB* (cytolethal distending toxin B), *ireA* (siderophore receptor), *kpsM* III (group 3 capsule), K1 and K2 *kpsM* II variants, *cvaC* (microcin V), *ibeA* (invasion of brain endothelium), and *rfc* (O4 lipopolysaccharide biosynthesis).
§Traits not detected in any isolate include F7–1 and F536 *papA* alleles and K15 *kpsM* II variant.
¶By Fisher exact test. Values are shown only where p≤0.01. HS, susceptible isolates from humans; HR, drug-resistant isolates from humans. Because drug-resistant and drug-susceptible poultry isolates showed no significant differences, they were combined into an all-poultry group.
#Negative association.

per sample was 1 for human fecal samples and 2 for poultry (range 1–4 for both).

Overall, 331 isolates (70 human, 261 poultry) were classified as resistant on the basis of reduced susceptibility to TMP-SMZ, quinolones/fluoroquinolones, and extended-spectrum cephalosporins. The remaining 600 isolates (460 human, 140 poultry) were susceptible to all these drug classes and were classified as susceptible (regardless of other possible drug resistance). The resistant isolates were distributed by resistance phenotype as follows: TMP-SMZ, 154 (47 human, 107 poultry); quinolones, 115 (26 human, 89 poultry); and extended-spectrum cephalosporins, 114 (14 human, 100 poultry). The 7 fluoroquinolone-resistant isolates (5 human, 2 poultry) were analyzed within the quinolone-resistant group.

Phylogenetic Distribution and Prevalence of ExPEC-defining Markers

The initial screening showed the 931 isolates to be fairly evenly distributed among the 4 major *E. coli* phylogenetic groups (20%–28% per group). However, they had various prevalences (2%–39% each) of the screening ExPEC virulence genes (Table 1). Overall, 27% of the isolates qualified as ExPEC by having ≥2 of the 5 ExPEC-defining markers (Table 1).

For enhanced resolution of similarities and differences, the 243 available ExPEC isolates underwent extended virulence genotyping for 60 ExPEC-associated virulence genes. All but 6 of these traits were detected in ≥1 isolate each, with prevalences ranging from 0.4% to 98% (Table 2).

Prevalence Comparisons

Phylogenetic group distribution and virulence gene prevalence differed considerably according to source (human versus poultry) and resistance status. This finding is shown in Table 1 for all 931 isolates (screening virulence genes only) and in Table 2 for the 243 ExPEC isolates (extended virulence profiles). Drug-resistant and drug-susceptible human isolates were separately compared with the combined group of all poultry isolates (i.e., all susceptible and resistant). We analyzed poultry isolates as a single group because the distribution of traits was similar in drug-resistant and susceptible poultry isolates; i.e., only 1 trait (*iutA*) was significantly associated with resistance among poultry isolates.

Consistent differences in phylogenetic and virulence gene distribution were evident between groups (Tables 1, 2). First, drug-susceptible human isolates differed considerably from drug-resistant human isolates. Second, drug-susceptible human isolates differed from poultry isolates. Third, although human drug-resistant isolates and poultry isolates exhibited some differences, these were considerably fewer and less extreme than those between drug-susceptible human isolates and poultry isolates. Similar results were obtained in subgroup analyses when isolates from hospital patient fecal samples were compared separately with isolates from conventionally raised poultry or when isolates from fecal samples from vegetarians were compared separately with isolates from poultry raised without antibiotics.

PCA

PCA was used to concurrently analyze multiple bacterial characteristics. The first PCA was conducted for the total population (n = 931) with the 7 screening virulence genes plus phylogenetic group. According to a 2 × 2 (source × resistance status) MANOVA of the first 2 axes of the PCA (which accounted for 65% of total variance), all 3 independent variables considered (source, resistance status, and interaction term) showed a p value ≤0.001. Accordingly, pairwise comparisons were made between individual source-resistance groups by 1-factor MANOVA. Susceptible human isolates differed ($p<0.001$) from each of the other 3 groups, whereas the other 3 groups differed marginally from each other. The individual axes supported this conclusion. These axes showed more extreme differences between drug-susceptible human isolates and each of the other 3 groups ($p<0.001$ for 5 of 6 comparisons) than among the other groups ($p>0.01$ for 4 of 6 comparisons).

Next, PCA was conducted for the 243 available ExPEC isolates based on all 60 virulence genes plus phylogenetic group. According to an initial 2 × 2 MANOVA of the results from the first 2 PCA axes (which accounted for 57% of total variance), all 3 independent variables (source, resistance status, and interaction term) showed a p value <0.001. Accordingly, pairwise comparisons were made between individual source-resistance groups by 1-factor MANOVA. Susceptible human isolates differed ($p<0.001$) from each of the other 3 groups, whereas the other 3 groups did not differ significantly from each another. In a plot of the (axis 1–axis 2) plane, drug-susceptible poultry isolates, drug-resistant poultry isolates, and drug-resistant human isolates overlapped and were confined largely to the left half of the grid (negative values on axis 1). In contrast, drug-susceptible human isolates, although overlapping somewhat with these groups, were concentrated principally within the right half of the grid (positive values on axis 1) (Figure 1).

Aggregate Virulence Scores

The various source and resistance groups were also compared for aggregate virulence scores (ExPEC isolates only). According to virulence score distribution, drug-susceptible human isolates (higher scores) segregated widely from the other 3 subgroups (lower scores), which were largely superimposed on each another (Figure 2). Because drug-resistant and drug-susceptible poultry isolates had similar virulence scores, they were combined for statistical

Table 1. Bacterial traits by source and antimicrobial drug resistance in 931 *Escherichia coli* isolates from human feces and poultry products, Minnesota and Wisconsin, 2002–2004*

	Prevalence, no. (%)				p value‡		
Trait†	Total (n = 931)	Human, susceptible (n = 460)	Human, resistant (n = 70)	Poultry (n = 401)	HS vs. HR	HS vs. all poultry	HR vs. all poultry
Group A	252 (27)	96 (21)	23 (33)	133 (33)		≤0.001	
Group B1	186 (20)	79 (17)	11 (16)	96 (24)			
Group B2	234 (25)	178 (39)	13 (19)	43 (11)	≤0.001	≤0.001	
Group D	259 (28)	107 (23)	23 (33)	129 (32)		≤0.01	
papA	124 (13)	98 (21)	6 (9)	20 (5)		≤0.001	
papC	163 (18)	100 (22)	10 (14)	53 (13)		≤0.001	
sfa/focDE	69 (7)	65 (14)	2 (3)	2 (0.5)	≤0.01	≤0.001	
afa/draBC	19 (2)	14 (3)	5 (7)	0 (0)		≤0.001	≤0.001
iutA	361 (39)	93 (20)	32 (46)	236 (59)	≤0.001§	≤0.001§	
kpsM II	288 (31)	195 (42)	23 (33)	70 (17)		≤0.001	≤0.01
hlyD	71 (8)	64 (14)	2 (3)	4 (1)	≤0.01	≤0.001	
ExPEC	249 (27)	147 (32)	20 (29)	82 (20)		≤0.001	

*Data are for the total population. Susceptible, susceptible to trimethoprim-sulfamethoxazole, nalidixic acid (quinolones), and ceftriaxone or ceftazidime (extended-spectrum cephalosporins), regardless of other possible drug resistance; resistant, resistant to 1 of the following: trimethoprim-sulfamethoxazole, nalidixic acid (quinolones), and ceftriaxone or ceftazidime (extended-spectrum cephalosporins).
†Groups A, B1, B2, and D, major *E. coli* phylogenetic groups; *papA* and *papC*, P fimbriae structural subunit and assembly; *sfa/focDE*, S and F1C fimbriae; *afa/draBC*, Dr binding adhesins; *iutA*, aerobactin system; *kpsM* II, group 2 capsule; *hlyD*, α-hemolysin; ExPEC, extraintestinal pathogenic *E. coli* defined by presence of ≥2 of *papA* and/or *papC* (counted as 1), *sfa/focDE*, *afa/draBC*, *iutA*, and *kpsM* II.
‡By Fisher exact test. Values are shown only where p≤0.01. HS, susceptible isolates from humans; HR, resistant isolates from humans. Because drug-resistant and drug-susceptible poultry isolates showed only 1 significant difference (for *iutA*), they were combined into an all-poultry group.
§Negative association.

ExPEC if ≥2 of the following were present: *papA* and/or *papC* (P fimbriae structural subunit and assembly), *sfa/focDE* (S and F1C fimbriae), *afa/draBC* (Dr binding adhesins), *iutA* (aerobactin system), and *kpsM* II (group 2 capsule) (*8*). All ExPEC isolates were then tested for 60 ExPEC-associated virulence genes and alleles thereof. Testing was conducted by using 2 independently prepared lysates of each isolate and established PCR-based methods (*12*,*17*). Isolates from various source groups (e.g., hospital volunteers, conventionally raised poultry) were tested in parallel to avoid cohort effects. The virulence score was the number of virulence genes detected adjusted for multiple detection of the *pap*, *sfa/foc*, and *kps* operons (*12*).

Statistical Methods

The unit of analysis was the individual isolate. Comparisons of proportions were tested by using Fisher exact test (2-tailed). Comparisons of virulence scores were tested by using Mann-Whitney U test (2-tailed exact probability). Principal coordinates analysis (PCA), also known as metric multidimensional scaling, is a multivariate statistical technique used to provide a simpler, low-dimensional graphic summary of the similarity between multiple samples (e.g., isolates) across multiple loci (*18*). New axes for plotting the isolates are derived from a data matrix of estimated dissimilarities between isolates. The first 2 principal coordinates, which account for the most variance, are used to plot the data. The distances between points in the plot represent isolate similarity. The dimensions represented by the (statistically uncorrelated) axes have no intrinsic meaning, i.e., they have no units. Using GenAlEx6 (*19*), we applied PCA to the screening dataset (all isolates) and the extended virulence profile dataset (ExPEC isolates) as a way to collapse the multiple variables for simplified among-group comparisons. For each PCA, results for each isolate from the first 2 PCA axes were used in multiple analysis of variance (MANOVA) to test for among-group differences. These values also were plotted to spatially represent the degree of separation or overlap of isolates on the 2-axis plane. For the ExPEC isolates, pairwise similarity relationships according to extended virulence profiles and phylogenetic group were used to construct a dendrogram according to the unweighted pair group method with arithmetic averages (*20*). The criterion for statistical significance throughout was p≤0.01 to account for multiple comparisons.

Results

Isolation of Drug-Resistant and Drug-Susceptible *E. coli*

Selective processing of 942 human fecal and poultry samples yielded 931 unique *E. coli* isolates, which constituted the study population. Of the 931 isolates, 530 (57%) were from human volunteers and 401 (43%) from poultry products. Of the human isolates, 456 (86%) were from hospital patients and 74 (14%) from vegetarians. Of the poultry isolates, 289 (72%) were from conventionally raised retail poultry and 112 (28%) from poultry raised without antibiotics. The median number of unique *E. coli* isolates

source resistant and susceptible isolates are similar, which is consistent with emergence of resistance on farms under selection from agricultural use of antimicrobial drugs.

Methods

Participants and Bacterial Strains

Human fecal samples were collected from 622 adults newly admitted to local hospitals in 4 rural communities in Minnesota (Willmar) or Wisconsin (Eau Claire, La Crosse, and Marshfield) and from 100 healthy self-identified vegetarians in these and nearby communities (*14*). Hospital patients were recruited from June 2002 through May 2003, vegetarians during the first 6 months of 2004. Fecal samples were collected by study personnel by using rectal swabs (hospital patients) or by the participants (vegetarians). To prevent isolation of hospital-acquired flora, inpatients samples were collected within 36 hours of hospital admission. Guidelines of the authors' institutions regarding use of human subjects were followed in this study. The relevant institutional review boards reviewed and approved the protocol. All participants provided informed consent.

A total of 180 retail poultry products (155 chicken and 25 turkey) were sampled (*14*). Conventional brands were purchased systematically from all food markets in the 4 primary study communities from May 2002 through May 2003, with 40 retail items obtained per community (total 160 items). These represented at least 18 plants in 11 states. Twenty samples with labels indicating that the poultry were raised naturally or without antibiotics were purchased in or near the study communities in August 2004. Additionally, 40 freshly slaughtered chicken carcasses from local farmers who raised chickens naturally or without antibiotics were obtained during plant inspections by the Minnesota Department of Agriculture from September 2003 through August 2004. The latter 2 groups of chickens, designated "no antibiotics," were confirmed to have been raised without antibiotics, based on the product label or by contacting the manufacturer or distributor.

Sample Processing

Human fecal samples were suspended and poultry samples and carcasses were massaged in nutrient broth, which was then incubated overnight at 37°C and stored as aliquots at –80°C in glycerol (*14*). Portions of these frozen stocks were transferred to vancomycin-supplemented (20 mg/L) Luria-Bertani broth. After overnight incubation, these broths were plated directly onto modified Mueller-Hinton (MMH) agar (Amyes medium) (*10*) with and without ciprofloxacin (4 mg/L) and (separately) nalidixic acid (32 mg/L), and were then incubated overnight. Samples of these Luria-Bertani broths containing vancomycin were placed in MMH broths supplemented individually with TMP-SMZ (4 mg/L TMP plus 76 mg/mL SMZ), cefoxitin (10 mg/L and 32 mg/L), and ceftazidime (10 mg/L and 32 mg/L). After overnight incubation, these broths were plated onto MMH agar plates supplemented with the corresponding agent (same concentrations) for overnight incubation. Colonies resembling *E. coli* were identified by using the API-20E System (bioMérieux, Marcy-l'Etoile, France).

Susceptibility Testing

At least 1 *E. coli* colony was randomly selected from each MMH agar plate and tested for disk susceptibility to 24 antimicrobial agents by using Clinical Laboratory Standards Institute (CLSI)–recommended methods, interpretive criteria, and reference strains (*15*). For isolates resistant to TMP-SMZ, nalidixic acid, or ciprofloxacin, the MIC was determined by Etest (AB-Biodisk, Sona, Sweden) according to the manufacturer's directions. Isolates from cefoxitin- and ceftazidime-supplemented plates underwent broth dilution MIC determinations with cefotaxime and ceftazidime regardless of disk test results. Isolates were classified as resistant to TMP-SMZ if the TMP MIC was ≥4 mg/L and the SMZ MIC was ≥76 mg/L, to quinolones if the nalidixic acid MIC was ≥32 mg/L, to fluoroquinolones if the ciprofloxacin MIC was ≥4 mg/L, and to extended-spectrum cephalosporins if the MIC to either cefotaxime or ceftazidime was ≥16 mg/L. The latter threshold corresponds with intermediate susceptibility per CLSI criteria and includes isolates with potentially clinically relevant reduced susceptibility. Because of the small number of isolates within each resistance phenotype, isolates were classified as resistant if they met any of these resistance criteria. Isolates that did not meet any of these resistance criteria were classified as susceptible, even though they may have had reduced susceptibility to other drug classes.

From each sample, 1 colony of each resistance phenotype (TMP-SMZ, quinolones, fluoroquinolones, extended-spectrum cephalosporins) and 1 susceptible isolate, as available, were selected. If multiple isolates from a given sample exhibited similar disk diffusion susceptibility profiles, genomic profiles as generated by using random amplified polymorphic DNA (RAPD) analysis were compared in the same gel (*12*). One representative of each unique RAPD genotype (as determined by visual inspection) was arbitrarily selected for further analysis.

Phylogenetic Analysis and Virulence Genotyping

All isolates were categorized as to major *E. coli* phylogenetic group (A, B1, B2, or D) by a multiplex PCR-based assay (*16*) (Table 1). Genes encoding proven or putative virulence factors of extraintestinal pathogenic *E. coli* (ExPEC) were detected in a sequential fashion. All isolates were screened for 5 ExPEC-defining virulence genes and *hlyD* (hemolysin). Isolates were operationally defined as

Antimicrobial Drug–Resistant *Escherichia coli* from Humans and Poultry Products, Minnesota and Wisconsin, 2002–2004

James R. Johnson,*† Mark R. Sannes,*†[1] Cynthia Croy,*† Brian Johnston,*† Connie Clabots,*† Michael A. Kuskowski,*† Jeff Bender,‡ Kirk E. Smith,§ Patricia L. Winokur,¶# and Edward A. Belongia**

The food supply, including poultry products, may transmit antimicrobial drug–resistant *Escherichia coli* to humans. To assess this hypothesis, 931 geographically and temporally matched *E. coli* isolates from human volunteers (hospital inpatients and healthy vegetarians) and commercial poultry products (conventionally raised or raised without antimicrobial drugs) were tested by PCR for phylogenetic group (A, B1, B2, D) and 60 virulence genes associated with extraintestinal pathogenic *E. coli*. Isolates resistant to trimethoprim-sulfamethoxazole, quinolones, and extended-spectrum cephalosporins (n = 331) were compared with drug-susceptible isolates (n = 600) stratified by source. Phylogenetic and virulence markers of drug-susceptible human isolates differed considerably from those of human and poultry isolates. In contrast, drug-resistant human isolates were similar to poultry isolates, and drug-susceptible and drug-resistant poultry isolates were largely indistinguishable. Many drug-resistant human fecal *E. coli* isolates may originate from poultry, whereas drug-resistant poultry-source *E. coli* isolates likely originate from susceptible poultry-source precursors.

Acquired resistance to first-line antimicrobial agents increasingly complicates the management of extraintestinal infections due to *Escherichia coli*, which are a major source of illness, death, and increased healthcare costs (*1–4*). One suspected source of drug-resistant *E. coli* in humans is use of antimicrobial drugs in agriculture. This use presumably selects for drug-resistant *E. coli*, which may be transmitted to humans through the food supply (*5–7*). Supporting this hypothesis is the high prevalence of antimicrobial drug–resistant *E. coli* in retail meat products, especially poultry (*8–11*), and the similar molecular characteristics of fluoroquinolone-resistant *E. coli* from chicken carcasses and from colonized and infected persons in Barcelona, Spain, in contrast to the marked differences between drug-susceptible and drug-resistant source isolates from humans (*12*).

To further assess the poultry-human connection, we used molecular typing to characterize drug-resistant and drug-susceptible *E. coli* isolates from feces of human volunteers or newly hospitalized patients in Minnesota and Wisconsin and from poultry products sold or processed in the same region. Resistance phenotypes of interest include trimethoprim-sulfamethoxazole (TMP-SMZ), quinolones/fluoroquinolones, and extended-spectrum cephalosporins. These agents are used for treatment of human *E. coli* infections. These drugs (or congeners) are also used in poultry production (e.g., each year in the United States an estimated 1.6 billion broiler eggs or chicks receive ceftiofur [*13*]); *E. coli* isolates resistant to these drugs are found in poultry. We examined, according to phylogenetic group distribution and virulence gene profile, whether drug-resistant human isolates more closely resemble susceptible human isolates, which is consistent with acquisition of resistance within humans, or instead resemble poultry isolates, which is consistent with foodborne transmission of poultry-source organisms to humans. We also examined whether poultry-

*Minneapolis Veterans Affairs Medical Center, Minneapolis, Minnesota, USA; †University of Minnesota, Minneapolis, Minnesota, USA; ‡University of Minnesota, Saint Paul, Minnesota, USA; §Minnesota Department of Health, Saint Paul, MN; ¶University of Iowa, Iowa City, Iowa, USA; #Iowa City Veterans Affairs Medical Center, Iowa City, Iowa, USA; and **Marshfield Clinic Research Foundation, Marshfield, Wisconsin, USA

[1]Current affiliation: Park Nicollet Clinic, Saint Louis Park, MN, USA

intravenous drug saved his life.

These days, there are very few new drugs available or even in the discovery pipeline. That means we need to preserve the efficacy of the life-saving medicines we have now, antibiotics like penicillin that are the marvel of modern medicine but are now misused and overused in livestock operations on a massive scale.

We're proud that at Chipotle, you can eat pork from Russ' pasture knowing it was responsibly produced by a farmer who is also a good steward of antibiotics. Unfortunately, that isn't the case everywhere. At present, only a very small portion of America's meat is raised without reliance on antibiotics. Our businesses are proof that livestock producers and food-sellers can make the switch profitably while protecting the public health. But as current business practice demonstrates, voluntary measures alone aren't enough to protect the public's health. We need the FDA to do its job and take real action to regulate the use of antibiotics in the meat industry. Americans' lives depend on it.

Steve Ells is founder and co-CEO of Chipotle. Russ Kremer is a pork producer in Missouri.

Regulate the use of antibiotics on farm animals

By Steve Ells and Russ Kremer The Denver Post
Posted:

DenverPost.com

The food you enjoy shouldn't make you or other people sick. That's our philosophy, as the CEO of one of the country's largest restaurant chains and one of its important suppliers. We believe food should taste good. But more than that, it should be good for you, and should be produced in a way that's good for communities and the ecosystems on which we all depend.

For years, we've operated successful businesses — Chipotle Mexican Grill and Ozark Mountain Pork Cooperative — with those ideas in mind. That's why we're concerned about the widespread abuse of antibiotics on factory farms, where most of America's meat and poultry are raised. Shockingly, the federal Food and Drug Administration now reports that 80 percent of the antibiotics sold in the U.S. are used for food animal production and that 90 percent of that is not being used to treat sick animals. Instead, the drugs are given at low doses, day after day, to very large numbers of animals to speed up growth and prevent diseases associated with the poor living conditions in crowded confinement buildings.

This dangerous practice is a key culprit in producing "superbugs" — bacteria that are resistant to the antibiotics we rely on. That's why Chipotle sources the pork for our more than 1,400 restaurants from farms like Russ' — where pigs roam free in pasture and are never given antibiotics unless they're sick. The results speak for themselves: The pork products Russ and his fellow Missouri family farmers make taste delicious.

This week in Fort Collins, the FDA is holding meetings for producers on the subject of factory-farm-bred antibiotic resistance. As socially responsible business owners intimately involved in our nation's food chain, we urge the agency to comply with two federal court orders that require it to ban non-therapeutic uses of antibiotics unless manufacturers can prove those uses are safe. For reasons of political expediency, the FDA is currently fighting these rulings in court.

Proving such uses are safe will be difficult. For decades, scientists have documented how routinely feeding farm animals low doses of antibiotics breeds superbugs and changes the microbial landscapes of our world and our bodies. That makes sense: In livestock operations, low doses of antibiotics kill off weak bacteria, leaving room for resistant ones to thrive.

Animal production facilities might seem remote from many of us, but research is demonstrating that the superbugs they breed travel off the farm easily to the outside world — through air, in soil, in water, on workers, and on meat itself. In fact, more than half the samples of ground beef, ground turkey and pork chops collected from grocery stores by the federal National Antimicrobial Resistance Monitoring System in 2011 were contaminated with antibiotic-resistant bacteria, according to a recent government analysis.

We know just how dangerous such bacteria can be. In 1989, when Russ still operated his farm according to the conventional industrial model, he got a life-threatening antibiotic-resistant infection after a 700-pound boar sliced open his knee. The bacteria were identical to ones found in his pigs. Doctors tried half a dozen antibiotics, but none of them was strong enough to knock out his superbug infection. Only a new

© istockphoto.com/vladacanon

BETTER WAYS TO ENSURE THE SAFETY OF MEAT ARE AVAILABLE

The most important factors affecting the safety of meat are how the animal is raised and how the meat is handled from the processing plant to your table. By focusing on good hygiene and improved management practices, a livestock facility can dramatically reduce illness among livestock and, in turn, the need for antibiotics. A recent study showed that chicken raised organically—on farms where antibiotics are prohibited—had less *Salmonella* than chicken from conventional livestock facilities, and far less of the *Salmonella* was resistant to antibiotics.[20]

WHAT YOU CAN DO TO ADDRESS THE PROBLEM AND PROTECT YOUR FAMILY

Curbing inappropriate use of antibiotics is key to maintaining their effectiveness in humans and slowing the growing problem of antibiotic resistance. While this effort will require work on multiple fronts—including the policy arena and in the marketplace—there are several important steps you can take at home right now:

- **Reduce overall consumption of animal products** as an important way to reduce your exposure to antibiotics in food and your overall environmental impact.
- **When buying meat, poultry, and dairy, look for products from animals raised without the use of antibiotics.** To be sure of what you are buying look for products with these labels: USDA Certified Organic, American Grassfed Certified, Animal Welfare Approved, and Certified Humane. Animal products bearing these labels are third-party certified as coming from farms where non-therapeutic uses of antibiotics are prohibited.
- **Prepare foods safely at home.** Follow these USDA-recommended practices to reduce risks from pathogens: use separate cutting boards for meat; wash hands, knives, and surfaces often; always cook to proper internal temperatures, checking with a meat thermometer; and refrigerate meat before cooking and within two hours after.

[1] World Health Organization. 2011. *Tackling Antibiotic Resistance from a Food Safety Perspective in Europe.* Accessed at www.euro.who.int/__data/assets/pdf_file/0005/136454/e94889.pdf.

[2] World Health Organization. 2011. *Policy Package to Combat Antimicrobial Drug Resistance, 4D Reduce Use of Antimicrobials in Food Producing Animals.* Accessed at www.who.int/world-health-day/2011/presskit/whd2011_fs4d_subanimal.pdf.

[3] M. Mellon, C. Benbrook, K.L. Benbrook. 2001. *Hogging It! Estimates of Antimicrobial Abuse in Livestock.* Cambridge, MA: Union of Concerned Scientists Publications. Accessed at www.ucsusa.org/food_and_agriculture/science_and_impacts/impacts_industrial_agriculture/hogging-it-estimates-of.html.

[4] Centers for Disease Control and Prevention (US). *Get Smart: Know When Antibiotics Work.* Accessed at www.cdc.gov/getsmart.

[5] R.R. Robert, B. Hota, I. Ahmad, D. Scott II, S.D. Foster, F. Abbasi, et al. 2009. Hospital and societal costs of antimicrobial resistant infections in a Chicago teaching hospital: Implications for antibiotic stewardship. *Clinical Infectious Diseases.* 49: 1175-1184.

[6] PR Newswire press release. 2009. *Antibiotic-Resistant Infections Cost the U.S. Healthcare System in Excess of $20 Billion Annually.* Accessed at www.biomerieux-usa.com/servlet/srt/bio/usa/dynPage?open=USA_NWS_NWS&doc=USA_NWS_NWS_G_PRS_RLS_73&crptprm=ZmlsdGVyPQ.

[7] Hearing: Antibiotic Resistance and the Use of Antibiotics in Animal Agriculture, Subcommittee on Health, Energy and Commerce Committee, US. House of Representatives. July 12, 2010. Accessed at www.energycommerce.house.gov/hearings/hearingdetail.aspx?NewsID=8001.

[8] World Health Organization. 2009. *Critically Important Antimicrobials for Human Medicine.* Accessed at www.who.int/foodsafety/foodborne_disease/CIA_2nd_rev_2009.pdf.

[9,10,12,15,16] E.K. Silbergeld, J. Graham, L. Price. 2008. Industrial food animal Production, antimicrobial resistance, and human health. *Annual Review of Public Health.* 29:151-69.

[11] R.I. Mackie, S. Koike, I. Krapac, J. Chee-Sanford, S. Maxwell, R.I. Aminov. 2006. Tetracycline residues and tetracycline resistance genes in groundwater impacted by swine production facilities. *Animal Biotechnology.* 17: 157-76.

[13] J. Burgos, B. Ellingon, M. Varela. 2005. Presence of multi-drug resistant enteric bacteria in dairy farm topsoil. *Journal of Dairy Science.* 88:1391-98.

[14] S.B. Levy, G.B. FitzGerald A.B. Macone. 1976. Changes in intestinal flora of farm personnel after introduction of a tetracycline-supplemented feed on a farm. *New England Journal of Medicine.* 295 (11): 583-588.

[17] Food and Drug Administration (US). 2009. National Antimicrobial Resistance Monitoring System 2009 Executive Report - Non-Typhoidal Salmonella Data, Table 22. Accessed at www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/ucm268962.htm

[18] A.E. Waters, T. Contente-Cuomo, J. Buchhagen, C.M. Liu, L. Watson, K. Pearce, et al. 2011. Multi-drug resistant Staphylococcus aureus in US Meat and Poultry. *Clinical Infectious Diseases.* 52: 1227-1230.

[19] K. Mattick, K. Durham, G. Domingue, F. Jørgensen, M. Sen, D.W. Schaffner, and T. Humphrey. 2002. The survival of foodborne pathogens during domestic washing-up and subsequent transfer onto washing-up sponges, kitchen surfaces and food. *International Journal of Food Microbiology.* 85: 213-226.

[20] W.Q. Alali, S. Thakur, R.D. Berghaus, M.P. Martin, W.A. Gebreyes. 2010. Prevalence and distribution of Salmonella in organic and conventional broiler poultry farms. *Foodborne pathogens and disease.* 7: 1364-1371.

www.nrdc.org/policy

NRDC

Food

Antibiotic resistant bacteria have been found on food crops irrigated with animal-waste-contaminated water, and many studies show a multitude of resistant organisms on meat and poultry products.[16] For example, the latest National Antimicrobial Resistance Monitoring System Executive Report shows that 48 percent of tested chicken breasts and 26 percent of tested ground turkey samples contaminated with *Salmonella* contained strains of the bacteria resistant to three or more types of antibiotics.[17] A recent study of meat and poultry from five U.S. cities found *Staphylococcus aureus* on 47 percent of samples. Ninety-six percent of those samples were resistant to at least one antibiotic, and 52 percent were multi-drug resistant.[18] Bacteria on food are carried into the kitchen where other foods can be cross-contaminated. One study simulated a typical wash-up in the kitchen using plates dirtied with food and contaminated with bacteria and found that a typical wash-up leaves plates, kitchen sponges, and countertops contaminated with bacteria.[19]

World Health Organization Classification of Antibiotics Used in People and Food Animals

Class of Antibiotics	Antibiotics in this Class for Human Use	Animal Antibiotic and Use	Most Commonly Used to Treat These Human Ailments	Particularly Important for Treating These Human Infections
Critically Important Antibiotics*				
Tetracyclines	Doxycycline, Minocycline, Tetracycline	Chlortetracycline: cattle, poultry, swine	Stomach ulcers caused by bacteria; acne	Chlamydia (a venereal disease); Rickettsial infections
Macrolides	Azithromycin, Clarithromycin, Erythromycin	Erythromycin and Tylosin: cattle, poultry, swine	Pneumonia; conjunctivitis (pink eye)	Legionnaire's Disease (a common form of pneumonia); Food poisoning, due to Campylobacter or multi-drug resistant *Salmonella*
Penicillins	Penicillin, Amoxicillin Amoxicillin/clavulanate potassium Piperacillin/tazobactam	Penicillin: poultry, swine	Strep throat; ear infections; sinus infections	Pseudomonas (a hospital superbug); Listeria (a dangerous infection for pregnant women and people with immune deficiency); Syphilis (a venereal disease)
Streptogramins	Quinupristin/Dalfopristin	Virginiamycin: cattle, poultry, swine	A variety of bacterial infections	Urinary tract infections; heart infection; meningitis due to resistant Enterococcus; Skin and blood stream methicillin–resistant *Staph. aureus* (MRSA) infections
Aminoglycosides	Gentamicin Tobramycin Amikacin Streptomycin	Gentamicin: swine; Spectinomycin: poultry, swine	Eye infections; various other bacterial infections	Drug-resistant tuberculosis (a growing global threat), used in combination with other drugs; Heart infections with Enterococcus
Highly Important Antibiotics**				
Sulfonamides	Sulfamethoxazole/ trimethoprim	Sulfamethazine: cattle and swine; Sulfathiazole: swine	Urinary tract infections	May be one of limited therapies for meningitis and other infections in certain geographic areas
Important Antibiotics				
Lincosamides	Clindamycin	Lincomycin: poultry and swine	A variety of bacterial infections	Necrotizing fasciitis ("flesh-eating disease") due to certain bacteria; MRSA skin infections

* These antibiotics meet both of the criteria below.

** These antibiotics meet either one of the criteria below.

CRITERIA:

A) These antibiotics are the *only* or one of a *very few* options used to treat serious human disease.

B) The bacteria that these drugs target can also be transmitted from non-human sources (such as agricultural animals) or can acquire resistance genes from non-human sources (such as agricultural animals).

"The techniques of microbiology and new developments such as synthetic biology will be crucial in achieving this," he said. (Editing by Jason Webb)

May 11, 2013
HUFFPOST HEALTHY LIVING

Antibiotic Resistance Poses 'Catastrophic Threat' To Medicine, Says Britain's Top Health Official

Reuters | By Kate Kelland
Posted: 03/10/2013 11:10 pm EDT | Updated: 04/22/2013 3:20 pm EDT

REUTERS

By Kate Kelland

LONDON, March 11 (Reuters) - Antibiotic resistance poses a catastrophic threat to medicine and could mean patients having minor surgery risk dying from infections that can no longer be treated, Britain's top health official said on Monday.

Sally Davies, the chief medical officer for England, said global action is needed to fight antibiotic, or antimicrobial, resistance and fill a drug "discovery void" by researching and developing new medicines to treat emerging, mutating infections.

Only a handful of new antibiotics have been developed and brought to market in the past few decades, and it is a race against time to find more, as bacterial infections increasingly evolve into "superbugs" resistant to existing drugs.

"Antimicrobial resistance poses a catastrophic threat. If we don't act now, any one of us could go into hospital in 20 years for minor surgery and die because of an ordinary infection that can't be treated by antibiotics," Davies told reporters as she published a report on infectious disease.

"And routine operations like hip replacements or organ transplants could be deadly because of the risk of infection."

One of the best known superbugs, MRSA, is alone estimated to kill around 19,000 people every year in the United States - far more than HIV and AIDS - and a similar number in Europe.

And others are spreading. Cases of totally drug resistant tuberculosis have appeared in recent years and a new wave of "super superbugs" with a mutation called NDM 1, which first emerged in India, has now turned up all over the world, from Britain to New Zealand.

Last year the WHO said untreatable superbug strains of gonorrhoea were spreading across the world.

Laura Piddock, a professor of microbiology at Birmingham University and director of the campaign group Antibiotic Action, welcomed Davies' efforts to raise awareness of the problem.

"There are an increasing number of infections for which there are virtually no therapeutic options, and we desperately need new discovery, research and development," she said.

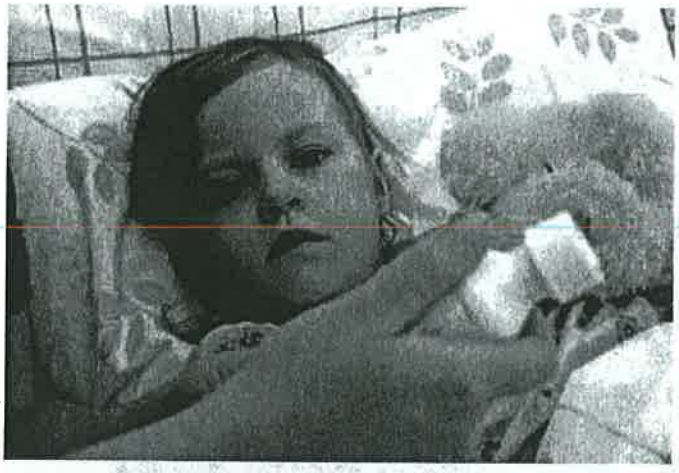
Davies called on governments and organisations across the world, including the World Health Organisation and the G8, to take the threat seriously and work to encourage more innovation and investment into the development of antibiotics.

"Over the past two decades there has been a discovery void around antibiotics, meaning diseases have evolved faster than the drugs to treat them," she said.

Davies called for more cooperation between the healthcare and pharmaceutical industries to preserve the existing arsenal of antibiotics, and more focus on developing new ones.

Increasing surveillance to keep track of drug-resistant superbugs, prescribing fewer antibiotics and making sure they are only prescribed when needed, and ensuring better hygiene to keep infections to a minimum were equally important, she said.

Nigel Brown, president of the Society for General Microbiology, agreed the issues demanded urgent action and said its members would work hard to better understand infectious diseases, reduce transmission of antibiotic resistance, and help develop new antibiotics.



© istockphoto.com/killerb10

WHY NON-THERAPEUTIC USE OF ANTIBIOTICS IS A PROBLEM

If you have an infection and receive a prescription for antibiotics, your doctor is likely to remind you to take all of the medication without missing any doses so that the treatment kills all of the bacteria responsible for the infection. If the antibiotic treatment is not sustained long enough at sufficiently high doses, antibiotic-resistant bacteria are more likely to survive and thrive. These bacteria can then multiply and pass on their drug-resistant traits to even more bacteria.

The same principle of antibiotic resistance applies in livestock. When animals are continually exposed to low levels of antibiotics, the bacteria that live in the gut, respiratory tract, and skin that are resistant to those particular antibiotics and other similar antibiotics are more likely to survive and flourish. These bacteria can then spread into the environment, through the movement of humans, animals, and animal products, to the livestock operator and local communities, to the consumer, and the public at large. The resistant bacteria multiply rapidly; some can even share the traits that make them drug-resistant with other species of bacteria, which enables bacteria that have never been exposed to antibiotics to become resistant, sometimes to multiple classes of antibiotics. These resistance traits can be carried silently in bacteria that can normally be found in and on the body. This helps lead to widespread drug-resistance and the emergence of bacterial superbugs, that resist all or most of the antibiotics available today to treat serious infections in humans.

THE DANGERS AND COSTS OF ANTIBIOTIC-RESISTANT INFECTIONS

According to the U.S. Centers for Disease Control and Prevention (CDC), antibiotic resistance is "one of the world's most pressing health problems."[4] A 2009 study from Cook County Hospital in Chicago and the Alliance for Prudent Use of Antibiotics showed that compared with non-resistant bacterial infections, resistant infections increase the risk of death two-fold and extend hospital stays by 6.4 to 12.7 days.[5] Extrapolating the data nationwide, antibiotic-resistant infections cost the U.S. healthcare system up to $26 billion annually, as consumers are continually exposed to antibiotic-resistant bacteria in meat and other food they purchase.[6]

PRESERVING THE EFFECTIVENESS OF ANTIBIOTICS FOR HUMAN USE

The CDC testified before Congress in July 2010 that the scientific evidence establishes "a clear link between antibiotic use in animals and antibiotic resistance in humans." The FDA and the U.S. Department of Agriculture (USDA) also acknowledged that the non-therapeutic use of antibiotics in food animals puts public health at risk.[7] Many leading health and medical groups, including the WHO, the American Medical Association, and the American Academy of Pediatrics, warn that the routine use of antibiotics in food animal production presents a serious and growing threat to human health because it contributes to the spread of dangerous antibiotic-resistant bacteria.

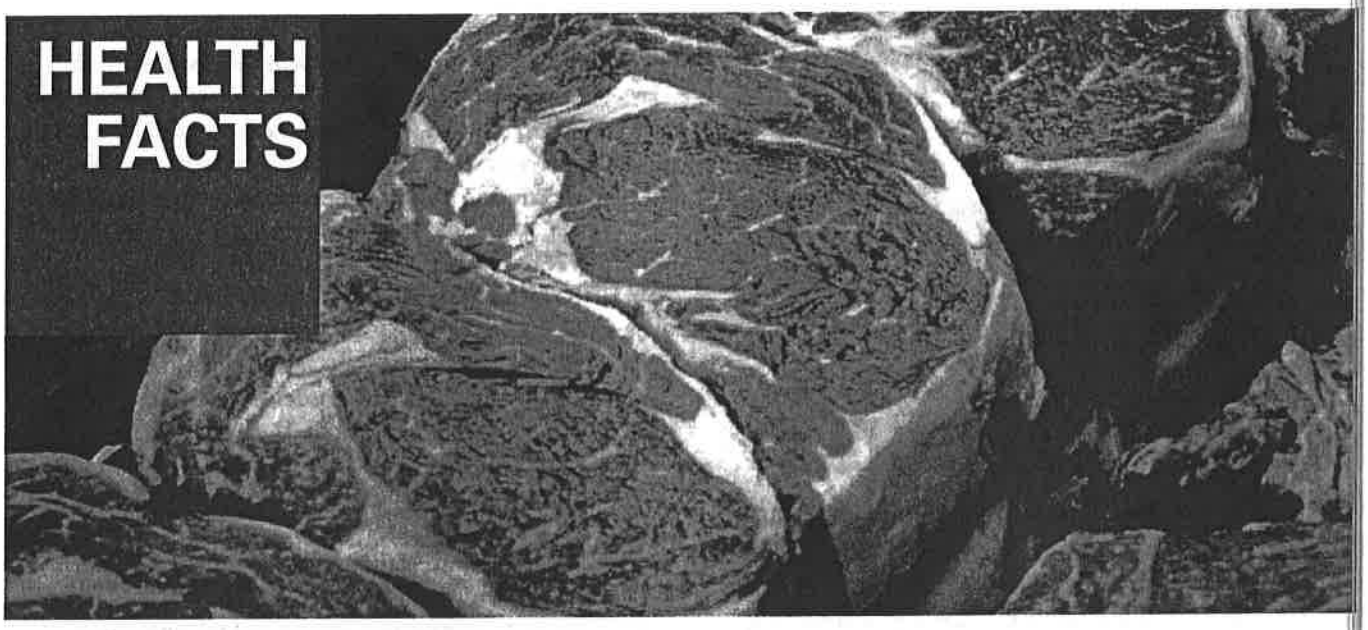
The WHO, together with the Food and Agricultural Organization and the World Organization for Animal Health, collaborated to classify antibiotics as "critically important," "highly important," or "important" for human use. Critically important antibiotics are either the *only* or one of a *very few* options available to treat serious human disease. Five classes of antibiotics used in agriculture in the United States are classified as "critically important" for human use, and one is "highly important" for human use (see table, World Health Organization Classification of Antibiotics Used in People and Food Animals).[8]

HOW ANTIBIOTIC-RESISTANT BACTERIA ENTER THE ENVIRONMENT AND AFFECT PEOPLE

Environmental Pathways

According to multiple studies, antibiotic resistant bacteria are found on and around industrial livestock facilities, in the soil, air, ground and surface waters, and in wild animal populations.[9] For example:

- *E. coli* with antibiotic resistance genes have been found in drinking water near hog facilities in three states;[10,11]
- antibiotic resistant bacteria have been detected in the air more than 160 yards downwind from industrial swine facilities;[12]
- multi-drug resistant bacteria have been detected in the topsoil of dairies;[13] and
- many studies demonstrate that resistant bacteria of livestock origin spread to farmers, who can then pass along the bacteria to their family, friends, and the public at large.[14,15]



Raising Resistance: Feeding Antibiotics to Healthy Food Animals Breeds Bacteria Dangerous to Human Health

Bacterial resistance to antibiotics is a major public health crisis. Increasingly, bacteria are resistant to multiple antibiotics, leading to infections that are difficult to treat and sometimes impossible to cure, require longer and more expensive hospital stays, and are more likely to be fatal. At the same time, the development of new antibiotics has slowed to a trickle. In some cases, there are now few or no antibiotics that work to treat drug-resistant bacterial infections. Meanwhile, scientific studies have shown that consumers are exposed to antibiotic-resistant bacteria on their meat and other food. While improper use of antibiotics in the health care sector is a problem, organizations such as the World Health Organization (WHO) recognize that the "overuse and misuse of antibiotics in food animals" is a major source of the antibiotic-resistant bacteria that affect humans, leading to infections that are harder to treat.[1]

THE GROWTH OF ANTIBIOTIC USE IN LIVESTOCK

In the 1950s, there were reports that healthy livestock grew faster if constantly fed low levels of antibiotics. Since then, it has become routine practice in many countries to incorporate low levels of antibiotics into the feed or water of healthy poultry, cattle, and swine to promote faster growth and prevent infections that tend to occur when animals are housed in crowded, unsanitary conditions. Often, the antibiotics used in U.S. agriculture are similar or identical to those used to treat human infections. This widespread use then leads to antibiotic-resistant bacteria, weakening physicians' arsenal of antibiotics effective for treating particular bacterial infections.

The WHO has called for the elimination of non-therapeutic uses of antibiotics such as for growth promotion and disease prevention, and has recommended antibiotic use in agriculture only by prescription by a veterinarian.[2]

The Food and Drug Administration (FDA), which regulates the use of antibiotics in food animals, recently confirmed that 80 percent of antibiotics sold in the United States are used not in humans but in food animals. Worse still, an estimated 83 percent of the antibiotics given to livestock in the United States are administered flock- or herd-wide at low levels without a prescription and for non-therapeutic purposes.[3]

NRDC
THE EARTH'S BEST DEFENSE

For more information, please contact:

Gina Solomon, MD, MPH
(415) 875-6100
gsolomon@nrdc.org
switchboard.nrdc.org/blogs/gsolomon

Avinash Kar
(415) 875-6122
akar@nrdc.org
switchboard.nrdc.org/blogs/akar

www.nrdc.org/policy
www.facebook.com/nrdc.org
www.twitter.com/nrdc

weaning at 28 days, initiatives concerning feed and proper care of sick animals.

Thus, institutional advantages have enabled Denmark to take ambitious risk mitigating strategies in order to combat antimicrobial usage and resistance – and without endangering the economic sustainability of the swine industry.

.....

In conclusion Denmark can state the following results:

- Antimicrobial resistance is reduced after the ban
- Total antibiotic consumption in food producing animals has been reduced by almost 40% from the mid 1990's till today
- Animal health has not been compromised
- Agricultural productivity has continued to improve
- The farmer's economy has not been significantly threatened
- Food safety in Danish products of animal origin has significantly improved as regards specifically Salmonella and Campylobacter
- A range of institutional factors helped Denmark implement the ban
- A ban on antibiotic growth promoters can be a very substantial and fulfilling first step in combating antimicrobial resistance, but should not stand alone in the long run

If you have any questions I will gladly answer them, and I will also direct our attention to the fact sheets handed out. Thank you for your attention.

tive - the 'yellow card' where farms using antibiotics above a certain threshold are mandated to reduce their use.

Salmonella levels have been between 0-2 % in eggs and chicken, and the Salmonella level in pork has remained low.

.....

When presenting the Danish experience here in the US, it is important to stress that Denmark is favoured by a range of institutional characteristics which helped implementing the ban and the following steps.

- In Denmark we can identify every herd, farmer and veterinarian and we are able to pinpoint the antimicrobial usage right down to the individual cow and to an age-group of swine. This is due to our many databases on husbandry and medicine usage. And we have also monitored and researched in resistance for the past 15 years in a targeted program called DANMAP.

- Our farming industry is highly organised in a co-operative structure with one common organisation for farmers and food companies. We have a longstanding tradition for working towards a consensus between government and industry and this was also the case with the ban on antimicrobial growth promoters.

- Working as an entity, the Danish swine industry has therefore played an important role and voluntarily stopped all non-therapeutic use of antibiotics, starting in 1998, with a total state ban in place by January 2000. Only two weeks ago the Danish swine industry again issued a voluntary ban; this time against therapeutic treatment with the critically important antibiotic Cephalosporin.

- Danish farmers are well educated and have easily learnt to produce pigs without antibiotic growth promoters. Instead they use good management,

duction from more than 75 % vancomycin resistance in enterococci isolated from broilers before the ban to less than 5% in 2006.

Additionally, Denmark has a markedly lower level of resistant bacteria in meat compared to imported meat from other EU member states. I can mention as an example, that the percentage of cephalosporin resistance in E. coli isolated from Danish broiler meat is less than 5%, while more than 35% of E. coli isolated from broiler meat from other EU-member states reveal cephalosporin resistance. This marked difference in resistance can be ascribed to our ban of growth promoters and low usage of antimicrobials compared to other EU countries. According to data from the European Food Safety Authority the total consumption of antimicrobials in food producing animals in 2007 was 120 metric tons in Denmark and almost 600 metric tons in another EU country with a comparable type of pig production.

The ban of growth promoters came into force in 1995 and we noted a substantial decrease of 40% in the consumption of antibiotics in the years thereafter.

The Danish swine industry has been producing pigs without the use of growth promoters for many years now and has increased both the production and the productivity. The same picture applies in the broiler chicken and cattle industries.

15 years after the ban the overall amount of antibiotics used for animals in Denmark is still almost 40% below the pre-ban level. As some US observers has pointed out, there has been an increase in the consumption of antimicrobials for therapeutical use during the post-ban years, but it has to be remembered, that the pig production has increased 25% in the same period, which can account for more than the increase in consumption of antimicrobials.

In the last few years and particularly in 2009 we have noted an increase of usage of antimicrobials above the concurrent increase in pig production. However, as this increase appears more than 10 years after the ban of growth promoters, we do not relate this to the ban. Nevertheless, we take this recent increase in usage seriously and have imposed the above-mentioned recent initia-

- Treatment guidelines for swine and cattle veterinary practitioners have been issued.

- Each individual veterinary practitioner is subjected to risk management and risk communication on prudent and reduced usage of antibiotics.

- Continuous monitoring and research in antimicrobial resistance in animals, humans and food.

- Monitoring of food borne pathogens in Danish as well as imported meat. Antimicrobial resistance is one of the parameters used to determine whether a shipment of food is dangerous.

- Control and action plans to combat Salmonella bacteria in poultry and pork and Campylobacter in poultry are all implemented

And the most recent development includes mandatory action plans in swineherds above a certain threshold value for antibiotics usage – the so called 'yellow card' initiative.

It is important to note that, according to our experience, a ban on antibiotic growth promoters can immediately and dramatically reduce the amount of antibiotics used. In Denmark the decrease was 40%. But such a ban should not stand-alone in the long run. This explains the fact that we have implemented this range of follow up measures and we expect also to have to take additional steps in the future.

.....

I would now briefly present some results of the initiatives:

The ban of growth promoters has resulted in a marked reduction in antimicrobial resistance as measured among several different bacterial species in food animals. The percentage of macrolide resistance in porcine Campylobacter has decreased from 80% before the ban to less than 20% in 2006. A similar re-

Treatment with antibiotics is in many cases essential for human and animal health and an uncritical use of antibiotics can lead to several antibiotics becoming ineffective.

Because antimicrobial resistance can be transferred between bacteria, regardless of whether the bacteria are pathogenic or not, the development of antimicrobial resistance in any kind of bacteria can constitute a problem.

It is a fact that antimicrobial resistance can be transferred from animals to humans by consumption of meat and every year also Denmark experience human outbreaks caused by consumption of meat, contaminated with antimicrobial resistant bacteria.

A ban on antimicrobial growth promoters was considered necessary for several reasons: There was science-based evidence that the use of antibiotics in animal feed could create resistance in pathogenic bacteria to medically important antibiotics, and there was a real concern that doctors would run out of options for treating life-threatening infections in humans.

Given the fact that very recently, a Danish PhD project concluded that production animals and meat might be a source of human E. coli urinary tract infections, the Danish ban seemed to be an example of due diligence.

.....

Among the initiatives, that are all mandated by the Danish government, I would like to mention the following:

- No prophylactic use of antimicrobials and mandatory low fixation of the veterinarians profit from sales of medicine.

- The critically important antibiotics fluoroquinolones can only be used, if a laboratory test shows, that no other antibiotics can be used.

Ministry of Food, Agriculture and Fisheries
Danish Veterinary and Food Administration

CHECK AGAINST DELIVERY

12 July 2010

Danish testimony on the July 14th Hearing about Antibiotic Resistance in the Livestock Industry organised by the Subcommittee on Health

By Per Henriksen, DVM, PhD, Head of Division for Chemical Food Safety, Animal Welfare and Veterinary Medicinal Products, The Danish Veterinary and Food Administration

Thank you, Mr. Chairman, Mr. Ranking Member, and Members of the Subcommittee, for inviting me to testify.

As a representative of the Danish government I am aware that the use of antibiotic growth promoters is a contentious issue here in the US and that Denmark is often mentioned in the debate. Against this background I wish to emphasize that the Danish government is not represented here today to advocate for or against any specific legislative proposals. However, we are an open nation, willing to share our experience when requested and therefore we have accepted your kind invitation.

I have submitted five fact sheets for the record, and with the Subcommittee's indulgence, I will therefore shorten my remarks to allow for your questions.

.....

Denmark is a major livestock producer in Europe, and the worlds' largest exporter of pork. The Danish livestock production is highly industrialised, intensive and applies modern management principles. Due to the significance for the Danish economy the National Government takes the competitiveness of the Danish farmers seriously.

their actions by exercising the same restraint shown by good doctors and patients: use antibiotics only by prescription for treatment or control of disease.

EWG recommends that consumers assume that all meat is contaminated with disease-causing bacteria. They can avoid superbugs in meat by eating less factory-farmed meat, by buying meat raised without antibiotics and by following other simple tips in EWG's downloadable **Tips to Avoiding Superbugs in Meat**.

For more information on the health and environmental consequences of various meats, see ewg.org/meateaterguide.

Make your voice heard! Click here to find out how you can help preserve the effectiveness of antibiotics.

risks that people will succumb to severe infection, hospitalization and death. In less than a decade, the proportion of antibiotic-resistant salmonella bacteria found on raw chicken has dramatically increased – from 48 percent in 2002 samples to **74 percent** in 2011 samples.

About 20 percent of the salmonella microbes detected on chicken samples collected in 2002 were resistant to at least three drugs. By 2011, that number had risen to **45 percent**. The proportion of antibiotic-resistant germs among all salmonella found on raw turkey rose from 62 percent in 2002 to **78 percent** in 2011.

SUPER CAMPYLOBACTER ON THE RISE

Campylobacter is one of the most common causes of diarrheal illness in the U.S. As well, it can trigger Guillain-Barre syndrome, an autoimmune disease that usually requires intensive care treatment and can lead to paralysis. *Campylobacter* germs cause 2.4 million foodborne illnesses and 124 deaths a year. The CDC reports that the rate of *Campylobacter* infections per 100,000 population increased by **14 percent** between 2006-2008 and 2011.

The most recent round of federal meat tests found that **26 percent** of raw chicken pieces contained an antibiotic-resistant form of *Campylobacter*. Of all the *Campylobacter* microbes found on the raw chicken samples, **58 percent** were resistant to at least one antibiotic, and **14 percent** were resistant to several antibiotics. Most alarmingly, all *Campylobacter* found on turkey were resistant to at least one antibiotic.

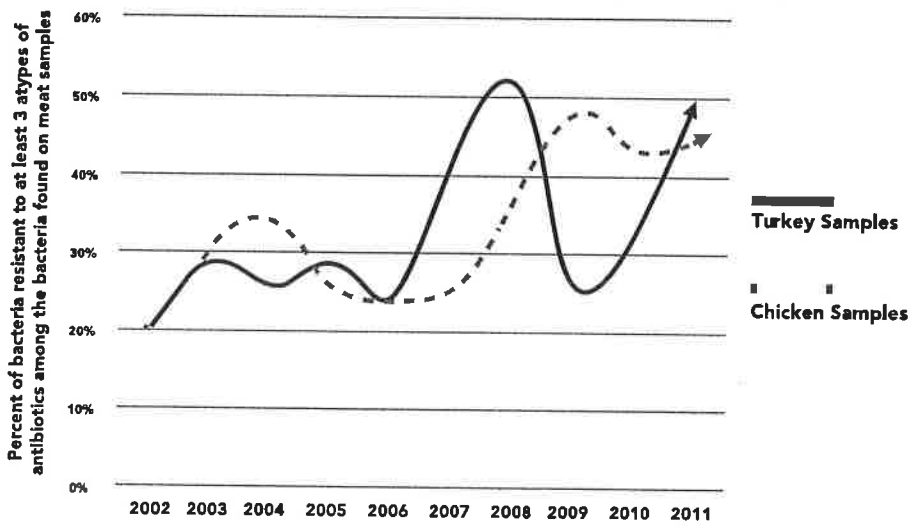
FIGHT SUPERBUGS

For more than 40 years, scientists and health experts have known that dangerous microbes were developing the ability to defeat valuable drugs. As far back as 1970 the FDA concluded that dosing livestock with unnecessary antibiotics spurred development of superbugs. Last year, the agency recommended that important antibiotics in farm animals "should be limited to those uses that are considered **necessary** for assuring animal health." It said that dosing animals with drugs solely to promote growth was "an injudicious use of these important drugs."

Nevertheless, the FDA's efforts to curb antibiotic abuse consist of only voluntary guidance documents – not regulations that carry the force of law. EWG takes the position that the FDA must take more aggressive steps to prevent superbugs from proliferating and livestock producers from squandering the effectiveness of vital medicines.

Big agribusinesses must take responsibility for

MULTI-DRUG-RESISTANT SALMONELLA IN POULTRY



Source: Chart prepared by EWG using data drawn from the National Antimicrobial Resistance Monitoring System's 2011 Retail Meat Report, published Feb. 5, 2013

MEAT SAMPLES TAINTED WITH INDICATOR BACTERIA

	TURKEY	PORK	BEEF	CHICKEN
Total samples tested	480	480	480	480
Number of samples contaminated with *Enterococcus faecalis*	392	334	269	186
Number of samples containing *Enterococcus faecalis* resistant to at least 1 antibiotic	389	332	263	185
Percent of meat samples containing antibiotic-resistant *Enterococcus faecalis*	**81%**	**69%**	**55%**	**39%**

Scientists study *Enterococcus* bacteria on meat to gauge fecal contamination and the spread of antibiotic-resistance traits.

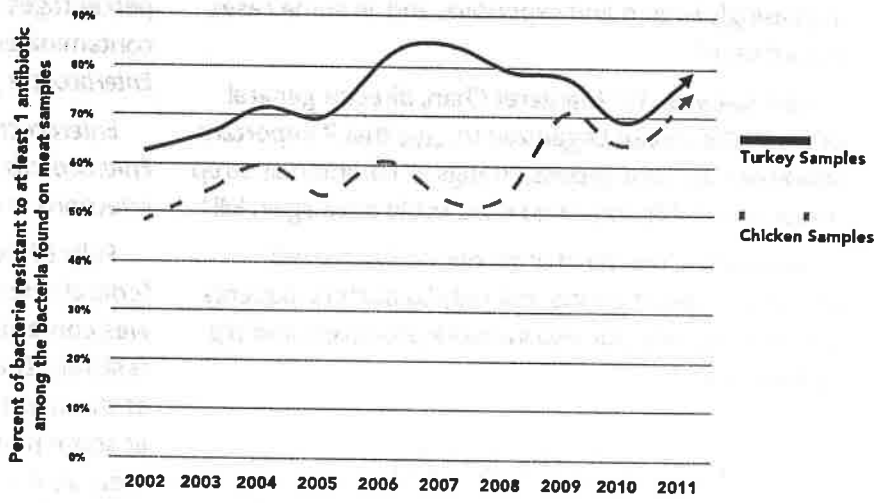
Source: EWG calculations based on data drawn from the National Antimicrobial Resistance Monitoring System 2011 Retail Meat Report, published Feb. 5, 2013

SUPER SALMONELLA ON THE RISE

Salmonella bacteria are often found on chicken and turkey that have been contaminated with animal feces. People can also encounter these microbes through cross-contamination – for instance, when salad greens are sliced on a cutting board that has been used to chop raw meat -- or by touching infected birds or reptiles. Infants have been known to contract salmonella by touching raw meat in a shopping cart. Salmonella-caused illnesses kill 400 people a year and cause 23,000 hospitalizations. They can lead to chronic arthritis.

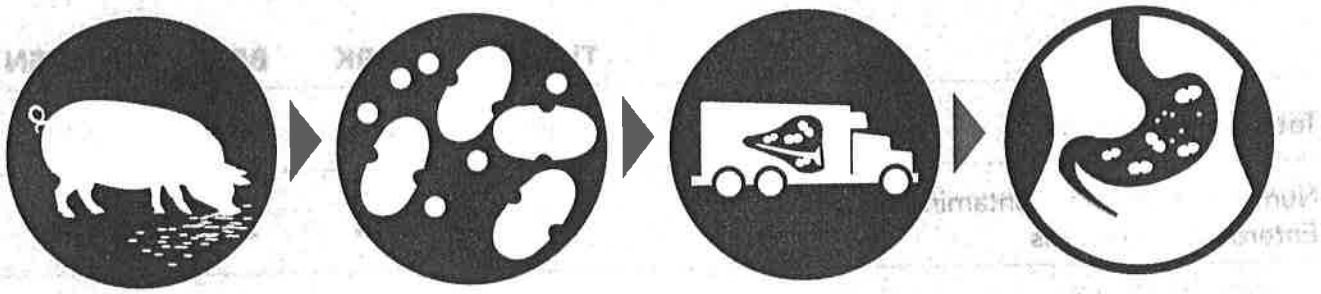
The rise of antibiotic-resistant salmonella has heightened the

ANTIBIOTIC-RESISTANT SALMONELLA IN POULTRY



Source: Chart prepared by EWG. EWG calculations based on data drawn from the National Antimicrobial Resistance Monitoring System's 2011 Retail Meat Report, published Feb. 5, 2013

SUPERBUGS FROM FARM TO FORK



Animals receive unnecessary antibiotics

Bacteria become resistant to antibiotics

Bacteria travel on meat from farm to stores

Meat may cause hard-to-treat illness

antibiotics market, according to the Pew Campaign on Human Health and Industrial Farming. Pew calculates that the market for antibiotics for treatment of people has been flat for some years, hovering at around 7.7 million pounds annually.

As the superbug problem has exploded into a full-fledged global health crisis, medical authorities worldwide are sounding increasingly urgent alarms.

The federal government's Interagency Task Force on Antimicrobial Resistance warned last year that "drug choices for the treatment of ... infections are becoming increasingly limited and expensive, and, in some cases, nonexistent."

Also last year, Dr. Margaret Chan, director general of the World Health Organization, said that if important antibiotics become useless, "things as common as strep throat or a child's scratched knee could once again kill."

Slowing the spread of antibiotic resistance will require concerted efforts, not only by doctors, patients and veterinarians but also livestock producers and big agribusinesses.

SUPERBUGS IN MEAT

EWG's research has determined that the risk of bringing a superbug into a kitchen varies by type of meat and how it was raised. Some types of meats are more contaminated than others. The overall picture is disturbing.

In the most recent round of federal tests, scientists used *Enterococcus* bacteria, normally found in human and animal intestines, as a gauge. For one thing, their presence can indicate contact with fecal matter. For another, *Enterococcus* bacteria easily develop and transmit antibiotic resistance. Counting the number of antibiotic-resistant *Enterococcus* on a particular meat sample can signal that other microbes on the meat are also likely antibiotic-resistant.

The scientists determined that startlingly high percentages of store-bought meat samples were contaminated with antibiotic-resistant forms of *Enterococcus faecalis.*

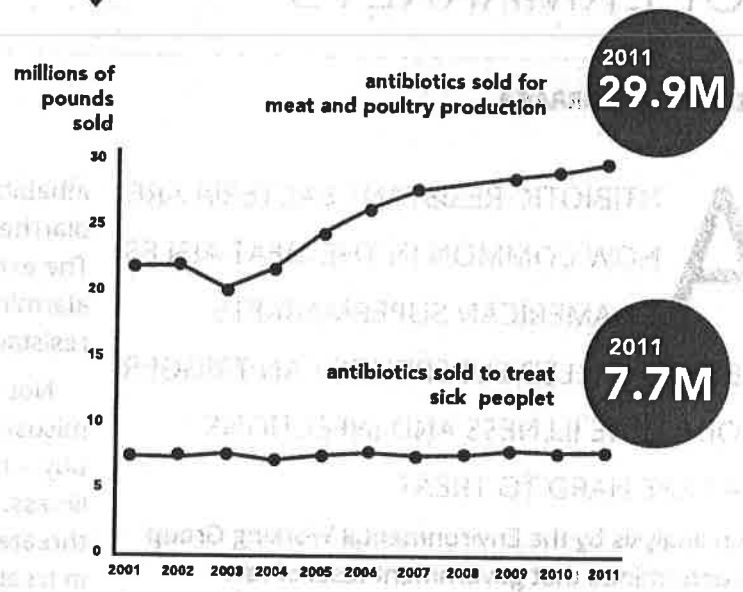
Enterococcus faecalis and the related species *Enterococcus faecium* are the third leading cause of infections in intensive care units of American hospitals.

Fully 87 percent of store-bought meat collected by federal scientists in the most recent round of tests was contaminated with both normal and antibiotic-resistant *Enterococcus* bacteria, evidence that most of this meat likely came in contact with fecal matter at some point. To be safe, consumers should treat all meat as if it may be contaminated, mainly by cooking thoroughly and using safe shopping and kitchen practices (see EWG's downloadable Tips to Avoiding Superbugs in Meat).

and in extreme cases can lead to arthritis.

- In the same federal tests, a superbug version of the *Campylobacter jejuni* microbe was detected on **26 percent** of raw chicken pieces. Raw turkey samples contained numerically fewer of these microbes, but **100 percent** of those examined were antibiotic-resistant. The *Campylobacter jejuni* pathogen is a common cause of diarrhea and in severe cases can trigger an autoimmune disease that results in paralysis and requires intensive care treatment.
- In 2006 FDA scientists found superbug versions of a particularly troublesome strain of *E. coli*, responsible for more than 6 million infections a year in the U.S., on **16 percent** of ground turkey and **13 percent** of chicken. Fully **84 percent** of the *E. coli* bacteria identified in these tests were resistant to antibiotics.
- In its own tests of raw pork, published last January, Consumer Reports magazine found that **63 percent** contained a superbug version of *Yersinia enterocolitica*, a microbe that can cause long-lasting bouts of diarrhea.
- In 2011 tests, researchers at Northern Arizona University and the Translational Genomics Research Institute found that **74 percent** of store-bought raw turkey samples were tainted with *Staphylococcus aureus* bacteria resistant to at least one antibiotic. Of these staph bacteria, **79 percent** were resistant to **three or more** types of antibiotics. Staph can cause skin infections in exposed cuts or produce toxins that cause foodborne illness.

MOST DRUGS GO TO LIVESTOCK



Source: Pew Charitable Trusts. 2013. Record-high antiblotic sales for meat and poultry production. Available: http://www.pewhealth.org/other-resource/record-high-antibiotic-sales-for-meat-and-poultry-production-85899449119

A significant contributor to the looming superbug crisis, according to scientists and health experts, is **unnecessary** antibiotic usage by factory farms that produce most of the 8.9 billion animals raised for food in the U.S. every year. Industrial livestock producers routinely dose their animals with pharmaceuticals, mostly administered with limited veterinary oversight and frequently without prescriptions, to encourage faster growth or prevent infection in crowded, stressful and often unsanitary living conditions.

Overuse of antibiotics in people, often for colds and other viral illnesses, has contributed to antibiotic resistance, too, but responsible doctors generally take care not to prescribe them unnecessarily.

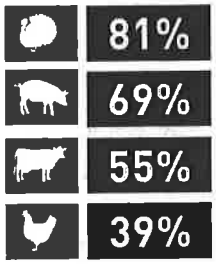
Pharmaceutical makers have powerful financial incentives to encourage abuse of antibiotics in livestock operations. In 2011, they sold nearly 30 million pounds of antibiotics for use on domestic food-producing animals, up 22 percent over 2005 sales by weight, according to reports complied by the FDA and the Animal Health Institute, an industry group. Today, pharmaceuticals sold for use on food-producing animals amount to nearly 80 percent of the American

SUPERBUGS INVADE AMERICAN SUPERMARKETS

BY DAWN UNDURRAGA

ANTIBIOTIC-RESISTANT BACTERIA ARE NOW COMMON IN THE MEAT AISLES OF AMERICAN SUPERMARKETS. THESE SO-CALLED SUPERBUGS CAN TRIGGER FOODBORNE ILLNESS AND INFECTIONS THAT ARE HARD TO TREAT.

An analysis by the Environmental Working Group has determined that government tests of raw supermarket meat published last February 5 detected antibiotic-resistant bacteria in:



These little-noticed tests, the most recent in a series conducted by the National Antimicrobial Resistance Monitoring System, a joint project of the federal Food and Drug Administration, Centers for Disease Control and Prevention and U.S. Department of Agriculture, found that supermarket meat samples collected in 2011 harbored significant amounts of the superbug versions of salmonella and *Campylobacter*, which together cause 3.6 million cases of food poisoning a year.

Moreover, the researchers found that some 53 percent of raw chicken samples collected in 2011 were tainted with an antibiotic-resistant form of *Escherichia coli*, or *E. coli*, a microbe that normally inhabits feces. Certain strains of *E. coli* can cause diarrhea, urinary tract infections and pneumonia. The extent of antibiotic-resistant *E. coli* on chicken is alarming because bacteria readily share antibiotic-resistance genes.

Not surprisingly, superbugs spawned by antibiotic misuse -- and now pervasive in the meat Americans buy -- have become a direct source of foodborne illness. Even more ominously, antibiotic misuse threatens to make important antibiotics ineffective in treating human disease. In the past, people who became ill because of contact with harmful microbes on raw meat usually recovered quickly when treated with antibiotics. But today, the chances are increasing that a person can suffer serious illness, complications or death because of a bacterial infection that doctors must struggle to control.

The proliferation of antibiotic-resistant bacteria poses special dangers to young children, pregnant women, the elderly and people with weakened immune systems.

Among the most worrisome recent developments:

- The federal tests published in February determined that **9 percent** of raw chicken samples and **10 percent** of raw ground turkey sampled from retail supermarkets in 2011 were tainted with a superbug version of salmonella bacteria. Antibiotic resistance in salmonella is growing fast: of all salmonella microbes found on raw chicken sampled in 2011, **74 percent** were antibiotic-resistant, compared to less than 50 percent in 2002. These microbes, frequently found on chicken and turkey and occasionally on beef and pork, commonly cause diarrhea

Contents

3 Introduction
5 Superbugs in meat
6 Super salmonella on the rise
7 Super *Campylobacter* on the rise
7 Fight superbugs

Acknowledgements
The authors thank Katie Clark and Lisa Frack for their assistance.

We would also like to thank Applegate who helped make this guide possible through an educational grant that we used to produce this guide.

The opinions expressed in this report are those of the authors and do not necessarily reflect the views of funders or reviewers."

HEADQUARTERS 1436 U Street. NW, Suite 100 Washington, DC 20009
(202) 667-6982
CALIFORNIA OFFICE 2201 Broadway, Suite 308 Oakland, CA 94612
MIDWEST OFFICE 103 E. 6th Street, Suite 201 Ames, IA 50010
SACRAMENTO OFFICE 1107 9th Street, Suite 625 Sacramento, CA 95814

ENVIRONMENTAL WORKING GROUP

www.ewg.org

Researchers
Dawn Undurraga
Johanna Congleton
Renee Sharp

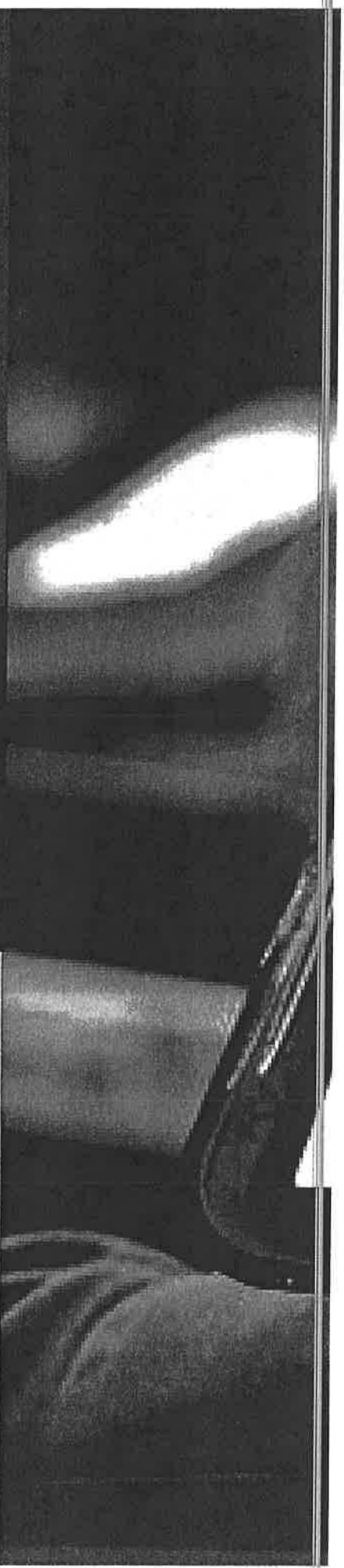
Content Reviewers
Kari Hamerschlag
Brett Lorenzen
Andrew Hug

Editors
Elaine Shannon
Nils Bruzelius

Designers
Aman Anderson
Ty Yalniz

About EWG
The mission of the Environmental Working Group (EWG) is to use the power of public information to protect public health and the environment. EWG is a 501(c)(3) non-profit organization, founded in 1993 by Ken Cook and Richard Wiles.

SUPERBUGS INVADE AMERICAN SUPERMARKETS



ENVIRONMENTAL WORKING GROUP

APRIL 2013

Germ count

Levels of contamination in the U.S. is about 50 pounds per year, based on 2009 Department of Agriculture data.

We tested 148 samples of meat from pork chops and 50 from ground pork, and found that almost 70 percent tested positive for yersinia enterocolitica, which can infect people who eat raw or undercooked pork.

Enterococcus, staphylococcus aureus, salmonella, and listeria monocytogenes were less common in the samples we tested. Twenty-three percent of the samples harbored none of the tested bacteria.

The pork samples we analyzed came from many brands, but we lacked enough samples within each brand to say whether one was more or less contaminated than another.

Big brands we tested: Denmark, Farmer John, Farmer John California Natural, Farmland, Hempler's, Hormel, Hormel Natural Choice, Nature's Promise, Nature's Rancher, Northwest Finest, Roseland, Smithfield, Swift Premium, and Tender Choice.

Store brands we tested: Angelo Caputo's, Bashas', Bristol Farms, Butera, Dominick's, Edmar, El Toreo Market, Food 4 Less, Fred Meyer, Fresh & Easy, The Fresh Market, Giant, Meijer, PCC Natural Markets, Publix, Ralphs, Safeway, Save-a-Lot, Sprouts Farmers Market and Farmers Market Old Tyme, Ultra Foods, Viet Wah, Vons, Walmart, Wegmans, Weis, Whole Foods, and Winn Dixie.

Levels of contamination

Bacterium	Samples testing positive
Yersinia enterocolitica	69%
Enterococcus	11
Staphylococcus aureus	7
Salmonella	4
Listeria monocytogenes	3

How resistant to antibiotics?

Some antibiotics used to treat infections in people are also fed to pigs to speed their growth or prevent illness. But bacteria may evolve to become immune to antibiotics, at which point the drugs become less effective in treating people infected by those bugs. We tested whether samples of salmonella, staphylococcus aureus, enterococcus, and yersinia enterocolitica that we isolated from pork chops and ground pork could survive exposure to up to 13 antibiotics at levels that are usually effective against those bacteria. The antibiotics we used differed with each bug but included amoxicillin, penicillin, tetracycline, streptomycin, and others.

Bugs immune to drugs

Bacterium	Samples tested	Samples resistant to one or more antibiotics	Details
Yersinia enterocolitica	132	121	Fifty-two of those were resistant to two or three antibiotics.
Staphylococcus aureus	14	13	Nine of those were resistant to two to four antibiotics.
Enterococcus	19	12	—
Salmonella	8	6	Three of those were resistant to five antibiotics.

Some 80 percent of all antibiotics sold in the U.S. are given to animals raised for food. Often, those drugs aren't used to treat infections but are fed continuously in low doses to promote growth and prevent infections that can spread in the cramped quarters in which most farm animals live. A single barn from a large hog-production facility can hold 2,000 or more pigs, creating ideal conditions for the spread of antibiotic-resistant bacteria.

"When you give low-dose antibiotics for growth promotion or for prophylaxis of infection, you end up killing off the susceptible bacteria, whether they're E. coli, salmonella, campylobacter, or other bacteria," says Robert S. Lawrence, M.D., director of the Center for a Livable Future at the Johns Hopkins Bloomberg School of Public Health in Baltimore. "And you continue to select for those bacteria that, through spontaneous mutations or transfer of genes from other resistant bacteria, allow them to be resistant to antibiotics." Lawrence cited recent laboratory research at Boston University suggesting that the continual exposure to low doses of antibiotics causes enough stress in bacteria to increase the rate of spontaneous mutations that render the bugs resistant to drugs, a process known as mutagenesis.

Mutant bacteria in animals can cause not only foodborne illness but also other treatment-resistant problems, such as infections of the skin or urinary tract. That's because the bugs don't just end up in the meat you buy; they can also wind up in fertilizer or contaminate the environment. And they can spread from person to person.

Another drug fed to animals, ractopamine, is given to as many as 60 to 80 percent of pigs raised in the U.S., by one estimate. It was originally developed (but never approved) as an asthma treatment for humans and was later found to boost pigs' growth and lean muscle mass.

The U.S. pork industry says ractopamine is safe. "Ractopamine is approved and used in 26 other countries, including some of the Asian countries," says Dave Warner, director of communications for the National Pork Producers Council, an industry group. "The issues with China and Taiwan have nothing to do with the safety of the product. Countries that have banned pork or meat from animals fed ractopamine are doing it to protect their domestic pork industries. This is not about food safety."

The European Food Safety Authority, which advises the European Union on food policy, concluded that it couldn't establish a safe level for ractopamine in food after reviewing the only study of its effect on humans (involving just six men). But it noted that drugs like ractopamine can cause restlessness, anxiety, a fast heart rate, and other conditions. And FDA documents show that it increases the risk of injury and lameness in pigs.

Warner emphasized that the U.S. pork industry uses ractopamine at levels that meet FDA and international food-safety standards. Indeed, although we found the drug at detectable levels in about 20 percent of our 240 pork samples, all had less than 5 parts per billion. That's well below the FDA's limit of 50 ppb in muscle tissue and the international limit of 10 ppb adopted in July 2012 by the Codex Alimentarius Commission, a program of the United Nations.

We asked three of the nation's largest pork producers—Smithfield Foods, Tyson, and JBS USA, which makes the Swift Premium and Swift Premium Natural brands—about their use of ractopamine. Keira Lombardo, vice president of investor relations and corporate communications at Smithfield, called it "a safe and effective FDA-approved feed supplement that has been widely used in the hog-farming industry for many years." Lombardo and a JBS spokeswoman, Margaret McDonald, told us their companies produce pork with and without ractopamine according to their customers' specifications.

Some food companies, including Chipotle Mexican Grill, Niman Ranch, and Whole Foods, say they don't sell any meat from pigs raised with ractopamine. Consumers Union, the policy and advocacy arm of Consumer Reports, has pressed for a ban of the drug, citing insufficient evidence that it's safe.

What you can do

These steps can help you minimize the risk of foodborne illness or discourage the routine use of antibiotics in agriculture:

Photo: Sean Gallup

- When cooking pork, use a meat thermometer to ensure that it reaches the proper internal temperature, which kills potentially harmful bacteria: at least 145° F for whole pork and 160° F for ground pork. (See our buying guide to meat thermometers.)
- As with other meats, keep raw pork and its juices separate from other foods, especially those eaten raw, such as salad.
- Wash your hands thoroughly after handling raw meat.
- Choose pork and other meat products that were raised without drugs. One way to do that is to buy certified organic pork, from pigs raised without antibiotics or ractopamine. Another option is to buy from Whole Foods, which requires that producers not use either type of drug.
- Look for a clear statement regarding antibiotic use. "No antibiotics used" claims with a USDA Process Verified shield are more reliable than those without verification. Labels such as "Animal Welfare Approved" and "Certified Humane" indicate the prudent use of antibiotics to treat illness.
- Watch out for misleading labels. "Natural" has nothing to do with antibiotic use or how an animal was raised. We found unapproved claims, including "no antibiotic residues," on packages of Sprouts pork sold in California and Arizona, and "no antibiotic growth promotants" on Farmland brand pork sold in several states. We reported those to the USDA in June 2012, and the agency told us it's working with those companies to take "appropriate actions." When we checked in early November, Sprouts had removed the claim from its packages. (See our guide to food labels.)
- If your local supermarket doesn't carry pork from pigs raised without antibiotics, consider asking the store to carry it. To find meat from animals that were raised sustainably—humanely and without drugs—go to eatwellguide.org. To learn about the Consumers Union campaign aimed at getting stores to sell only antibiotic-free meat, go to NotinMyFood.org.

ConsumerReports.org®

What's in that pork?

We found antibiotic-resistant bacteria and traces of a veterinary drug

Consumer Reports magazine: January 2013

Our analysis of pork-chop and ground-pork samples from around the U.S. found that yersinia enterocolitica, a bacterium that can cause fever, diarrhea, and abdominal pain, was widespread. Some samples harbored other potentially harmful bacteria, including salmonella. And there are more reasons to be concerned about "the other white meat."

Some of the bacteria we found in 198 samples proved to be resistant to antibiotics commonly used to treat people. The frequent use of low-dose antibiotics in pork farming may be accelerating the growth of drug-resistant "superbugs" that threaten human health.

About one-fifth of the 240 pork products we analyzed in a separate test harbored low levels of the drug ractopamine, which the U.S. approved in 1999 to promote growth and leanness in pigs. It's commonly used in pigs raised for food in the U.S. but is banned in the European Union, China, and Taiwan. Our food-safety experts say that no drugs should be used routinely in healthy animals to promote growth. Here are details from our tests:

- Yersinia enterocolitica was in 69 percent of the tested pork samples. It infects about 100,000 Americans a year, especially children.
- We found salmonella, staphylococcus aureus, or listeria monocytogenes, more common causes of foodborne illness, in 3 to 7 percent of samples. And 11 percent harbored enterococcus, which can indicate fecal contamination and can cause problems such as urinary-tract infections.
- Some of the bacteria we found were resistant to multiple drugs or classes of drugs. That's worrisome, because if those bugs make you sick, your doctor may need to prescribe more powerful (and expensive) antibiotics.
- Ground pork was more likely than pork chops to harbor pathogens. That's to be expected, since grinding meat provides another opportunity for contamination.
- Some antibiotic claims you'll see on packaging are misleading. And a "no hormones added" claim might be true but is meaningless, because hormones aren't allowed in pork production.

Read more about antibiotics in meat.

Did you know?
Years ago, trichinosis was the main fear about eating pork. But the risk from that parasite was largely eradicated by changes in industry practices (legislation banned the feeding of certain raw foods to hogs) and public awareness of the risks of eating undercooked meat.

Bugs in pigs

All animals (humans included) have bacteria on their skin and in their gastrointestinal tract. Some are beneficial, including the probiotic kind, which help digestion. Others, such as salmonella, can be harmful to people, but affected animals might not become ill. Confining animals in less-than-clean quarters can allow bad bacteria to proliferate.

An animal's muscles (meat), blood, and brain are normally sterile. But during slaughter and processing, meat can become contaminated with bacteria from the animal's skin or gut and from workers, equipment, or the environment. Contamination is especially likely to occur if processing lines run too fast or if sanitary practices aren't followed. Once bacteria are on meat, improper storage can encourage them to multiply.

To minimize contamination, the federal government requires processors of meat, poultry, and seafood to create safety and inspection procedures collectively known as HACCP (pronounced hass-ip), which stands for Hazard Analysis & Critical Control Points. Implemented for meat and poultry plants in 1997, HACCP is officially the consumer's first line of protection against contaminated pork. But inspectors spot-test for a limited number of pathogens. Yersinia enterocolitica, for example, isn't among them. And the Department of Agriculture can't require a recall if HACCP plans fail to meet goals.

"Very low contamination levels in hog carcasses indicate that companies' practices are adequately controlling pathogens," a USDA spokeswoman told us. But our tests showed that some harmful bacteria can make their way into your kitchen.

Moreover, the bacteria we found often continued to multiply even in the presence of some drugs designed to kill them or stop them from reproducing. Thirteen of 14 staphylococcus samples we isolated from pork were resistant to one or more antibiotics. So were six of eight salmonella samples, 12 of 19 enterococcus samples, and 121 of 132 yersinia samples. One sample was identified as MRSA, a drug-resistant and sometimes fatal staph.

Pigs on drugs

Kanamycin	17
Streptomycin	34
Cefoxitin	28
Ceftiofur	30
Ceftriaxone	0 (b)
Amoxicillin/clavulanic acid	28
Ampicillin	30
Chloramphenicol	2
Nalidixic acid	2
Sulfisoxazole	21
Tetracycline	49
One or more drugs	**68**

Campylobacter drug	**Resistant (c)**
Ciprofloxacin	18%
Nalidixic acid	21
Tetracycline	49
One or more drugs	**60**

(a) Tested drugs that were effective against salmonella: Amikacin, Ciprofloxacin, and Trimethoprim/Sulfamethoxazole. (b) 17% of samples were somewhat resistant: Ceftriaxone inhibited bacterial growth but didn't stop it. (c) Tested drugs that were effective against campylobacter: Gentamicin, Azithromycin, Erythromycin, Telithromycin, Clindamycin, and Florfenicol.

Cut-up and packaging area. Birds are cut into pieces if necessary, packaged, and shipped. Critical control point Check for metal fragments in packaged poultry.

Talk the talk

Certified Humane Raised and Handled. For starters, the chicken had access to clean food and water, according to third-party inspectors with expertise in animal welfare.

Free-range, free-roaming. The chicken has had access to the outdoors, even if that means only that the door to the chicken house was left open briefly each day.

Fresh. The carcass's internal temperature hasn't dropped below 24° F. Still, the chicken might be partly frozen.

Kosher. The chicken was prepared according to Jewish dietary laws. Salt was added as part of the process.

Natural. The chicken was "minimally processed" in a way that didn't fundamentally alter the raw product. It has no artificial ingredients, preservatives, or added color.

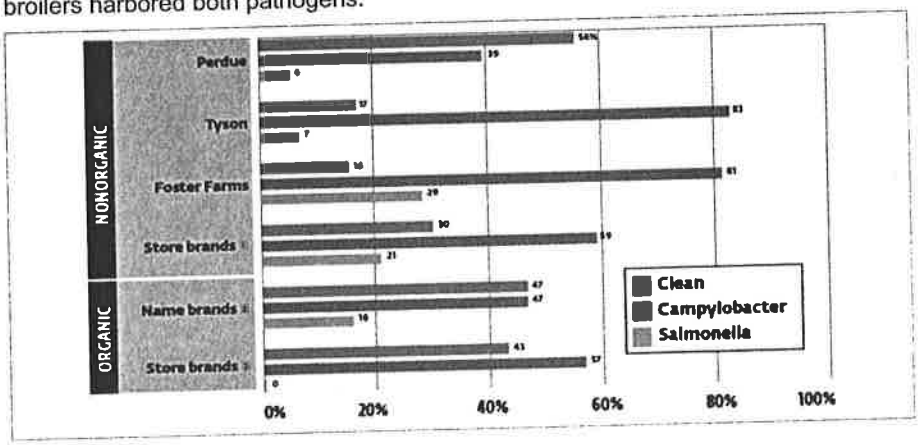
No antibiotics administered. Don't assume this was verified unless you also see the label "USDA organic."

No hormones. Pointless; the USDA prohibits the use of hormones in raising poultry.

USDA organic. A USDA-accredited certifier has checked that the chicken company followed standards: Chickens were raised without antibiotics, ate 100 percent organic feed with no animal byproducts, and could go outdoors (though they might not have). For more about labels, go to our affiliate Web site at *www.GreenerChoices.org*.

Levels of contamination

Below, the percentages of broilers that tested positive for campylobacter, salmonella, or neither (clean). We analyzed 70 chickens from each major brand, 66 from nonorganic store brands, 62 from organic name brands, and 44 from organic store brands. Figures are averages for store brands (both organic and nonorganic) and for organic name brands. Totals may exceed 100 percent because some broilers harbored both pathogens.



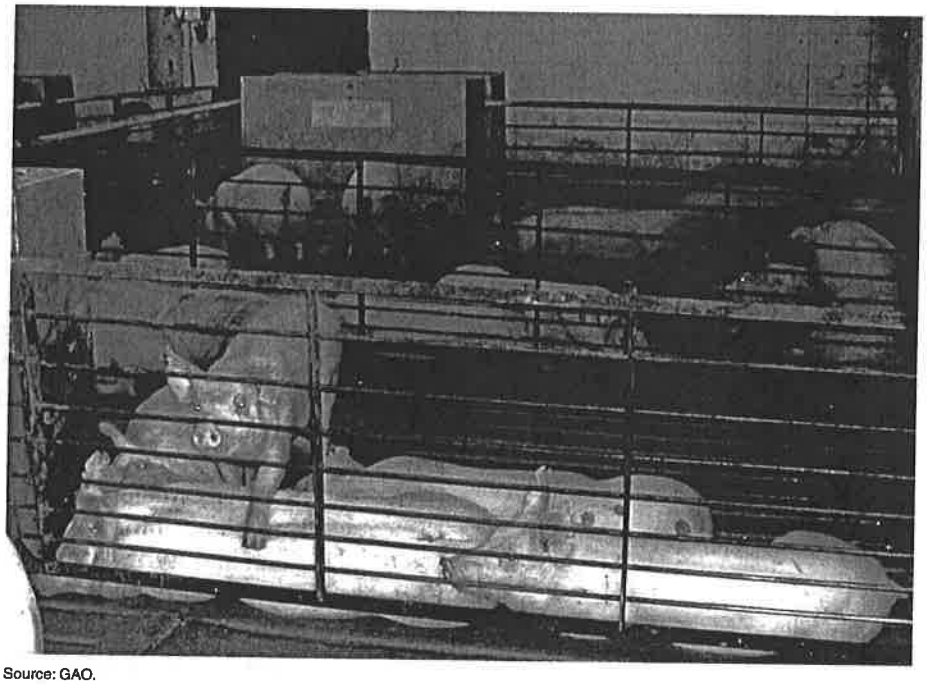
1. AJ's, Acme, Albertsons, America's Choice, Diebergs, Earth Fare, Fiesta, Fresh & Easy, Giant, Giant Eagle, Harris Teeter, Harry's, Hill Country Fare, Jewel, King Sooper, Kroger Value, Market Pantry, Nature's Promise, Publix, Roundy's, Safeway, Schnucks, Shaws, Shop 'n Save, Sweetbay, Tops, Wegmans, White Gem, Wild Harvest, Whole Foods.
2. Bell & Evans, Coastal Range, Coleman, D'Artagnan, Eberly's, MBA Brand Smart Chicken, Mary's, Pollo Rosso, Rosie.
3. Central Market HEB, O Organics (Safeway), Pacific Village (New Seasons), Private Selection Organic Fred Meyer, Private Selection Organic King Sooper, Private Selection Organic Kroger, Trader Joe's, Wegmans, Whole Foods.

Resistance to antibiotics

Some antibiotics important for humans are fed to nonorganic chickens to speed growth or prevent or treat illness. But bacteria may evolve to become immune to antibiotics, at which point the drugs become less effective in treating people. We took 53 salmonella samples and 103 campylobacter samples from chickens and determined what percentage of samples resisted antibiotics that usually work against those pathogens. "Resistant" indicates the percentage of bacteria that could survive a normal dose of the drug. Each color represents a class of antibiotics. Within classes, drugs are in alphabetical order.

Salmonella drug	Resistant (a)
Gentamicin	4%

Figure 1: Swine Confinement Facility



Source: GAO.

Possible Spread of Antibiotic-Resistant Bacteria from Animals to Humans

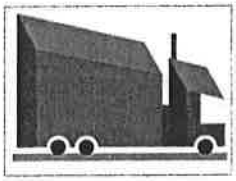
Figure 2 shows how antibiotic-resistant bacteria that develop in animals can possibly be transferred to humans, who may then develop a foodborne illness, such as a salmonella infection, that is resistant to antibiotic treatment.[11] Once the resistant bacteria develop in animals, they may be passed to humans through the consumption or handling of contaminated meat. An animal or human may carry antibiotic-resistant bacteria but show no signs or symptoms of an illness. Resistant bacteria may also be spread to fruits, vegetables, and fish products through soil, well water, and water runoff contaminated by waste material from animals harboring these bacteria, although such routes are beyond the focus of this report.

[11]Foodborne illnesses generally cause gastrointestinal symptoms, such as nausea, vomiting, abdominal cramps, and diarrhea. There are more than 250 foodborne diseases, and most are caused by bacteria, viruses, and parasites.

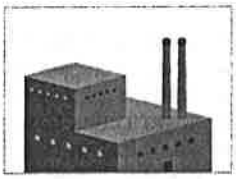
Abbreviations

ADG	average daily weight gain
ADP	antibiotics used for disease prevention
AGP	antibiotics used for growth promotion
CAHFSE	Collaboration in Animal Health, Food Safety, and Epidemiology
CDC	Centers for Disease Control and Prevention
DT	definitive type
DNA	deoxyribonucleic acid
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FAS	Foreign Agricultural Service
FCR	feed conversion ratio
FDA	Food and Drug Administration
FoodNet	Foodborne Diseases Active Surveillance Network
HIV	human immunodeficiency virus
HHS	Department of Health and Human Services
MR	mortality rate
NAHMS	National Animal Health Monitoring System
NARMS	National Antimicrobial Resistance Monitoring System—Enteric Bacteria
NRC	National Research Council
OIE	Office International des Epizooties
Q/D	quinupristin/dalfopristin
USDA	U.S. Department of Agriculture
WHO	World Health Organization
WTO	World Trade Organization

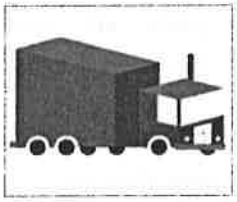
On the road
Chickens travel to the processing plant in cages. Filth can spread.



Processing plant
See In the processing plant.



After processing
Companies take steps to ensure their packaged chickens are properly refrigerated during shipping and delivery to market. Federal regulations require transport at a temperature no higher than 40° F.



In the store
Improper temperature or handling can introduce bacteria or cause them to multiply.

In your kitchen
Cooking chicken thoroughly, to at least 165° F, and washing anything that comes in contact with raw chicken greatly reduces risk.

In the processing plant
Birds are stunned, killed, and bled.

Scalding. Hot water loosens feathers for easier plucking. Some bacteria on feathers, feet, and skin are killed, but others float from one bird to another. Carcasses are washed. **Critical control point** Check temperature and pH of water.

Defeathering. A machine's rubber fingers pluck feathers and remove the outermost layer of skin. Contaminated fingers can spread bacteria from carcass to carcass.

USDA visual inspection. After internal organs are removed, a Department of Agriculture inspector checks carcasses and viscera for signs of disease, bruises, and other defects.

Washing. Birds are sprayed with chlorinated water or other washes to reduce bacteria and are checked for visible fecal matter. Chickens that pass muster are chilled; those that fail are reprocessed or discarded. **Critical control point** Record chlorine level and adjust if necessary.

Chilling. To prevent spoilage, carcasses are submerged in icy chlorinated water or air-chilled to lower their internal temperature to 40° F or less. When chickens emerge, USDA inspectors grade them for quality. At this stage, the USDA conducts salmonella testing, and the plant conducts one test for E. coli per 22,000 birds. Critical control point Monitor chlorine level of chiller or temperature of air-chill room; check internal temperature of birds.

What you can do

Too often, America's food-safety net has holes. Although Perdue chickens were cleaner than other big brands in our tests, and most air-chilled organic brands were especially clean, our tests are a snapshot in time and no type has been consistently low enough in pathogens to recommend over all others. Buying cleaner chicken may improve your odds if you fail to prepare chicken carefully. If you choose organic, be aware that it cost us up to $4.55 more per pound than the rest.

Whatever bird you buy, one slipup and you're at risk. Most important is to cook chicken to at least 165° F. Even if it's no longer pink, it can still harbor bacteria, so use a meat thermometer. The Polder THM-360, $30, and Taylor Weekend Warrior 806, $16, were excellent in our tests. Other tips:

- Make chicken one of the last items you buy before heading to the checkout line.
- Choose chicken that is well wrapped and at the bottom of the case, where the temperature should be coolest.
- Place chicken in a plastic bag like those in the produce department to keep juices from leaking.
- If you'll cook the chicken within a couple of days, store it at 40° F or below. Otherwise, freeze it.
- Thaw frozen chicken in a refrigerator, inside its packaging and on a plate, or on a plate in a microwave oven. Never thaw it on a counter: When the inside is still frozen, the outside can warm up, providing a breeding ground for bacteria. Cook chicken thawed in a microwave oven right away.
- Don't return cooked meat to the plate that held it raw.
- Refrigerate or freeze leftovers within 2 hours of cooking.

For more ways to help ensure that your food is safe, go to our Web site at *www.BuySafeEatWell.org*.

Sickened by chicken?

Within a few days of eating salad at a Minnesota restaurant in February 2009, Michele Lundell, a supervisor for a company that makes plastic tubing, experienced diarrhea, fever, and headache. "I kept getting sicker and sicker," she recalled. A test confirmed campylobacter. After her doctor prescribed antibiotics, Lundell said, she felt better for about a day, but then "all the same symptoms came back." She said she was hospitalized for six days. A Minnesota Department of Health investigation found that 10 people who had eaten at the restaurant were stricken with campylobacter and that the lettuce was most likely contaminated by raw or undercooked chicken. Lundell said she hasn't fully recovered. "It's hard to believe," she said, "that a person goes out to eat and gets so sick that it changes your life."

Michele Lundell, 53, of Apple Valley, Minn., became ill from campylobacter.

Science lesson: A little bit can make you sick

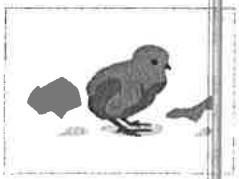
As few as 15 salmonella or 400 campylobacter organisms can make you ill. The salmonella found in raw poultry, meats, seafood, and produce can cause nausea, vomiting, abdominal cramps, diarrhea, fever, and headache, sometimes followed by arthritis symptoms. Campylobacter is found mainly in raw chicken. It wasn't recognized as a human pathogen until 1977, but it is now one of the most common causes of bacterial foodborne illness. The usual symptoms are diarrhea, often with fever, abdominal pain, nausea, headache, and muscle pain. Rarer are complications such as arthritis, meningitis, and Guillain-Barré syndrome, a potentially fatal neurological condition.

From henhouse to your house

The government's food-safety rules require chicken processors to identify "critical control points" where contamination might occur, then establish procedures for preventing, eliminating, or reducing those hazards. As our tests show, nothing guarantees a clean chicken. The contamination rate can vary with what the birds are fed, the preventive measures used, growing conditions, and the time of year, says Michael Doyle, Ph.D., director of the University of Georgia's Center for Food Safety. The procedures differ among plants; those outlined here are a possible scenario.

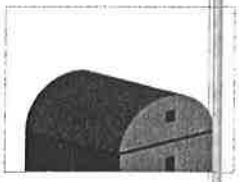
In the hatchery

Some chicks are contaminated with salmonella from their mothers or their own shells during hatching. Others ingest bacteria from their surroundings. Live birds infected with campylobacter or salmonella usually show no symptoms. To reduce the risk to people, some companies vaccinate hens and chicks against salmonella.



In the chicken house

Usually a new flock of thousands of chicks is trucked to a house run by a farmer according to the poultry producer's specifications. Chickens habitually peck the ground, ingesting bacteria from litter and feces, and could be exposed to vermin. Companies try to keep germ carriers away and continuously monitor the flocks' general health. Antibiotics are used to prevent or treat illness and might also be given to speed chickens' growth. But treated birds can't be sold as USDA-certified organic.



salmonella-positive samples over 51 consecutive days of production fail to meet the FSIS-established performance standard, which triggers an FSIS review of the plant's HACCP plan. The plant would be expected to fix any problems; penalties are possible. To further motivate chicken processors to clean up their act, the USDA has begun publicly posting processors' salmonella test results online. (The data isn't archived, making it hard to assess a processor's performance over time.)

With this gentle prodding, poultry plants have improved, FSIS data indicate. Yet only 82 percent of broiler plants demonstrate what the FSIS calls "consistent process control." By the end of 2010, 90 percent of eligible plants should be able to meet that standard, according to FSIS projections.

That still leaves campylobacter. As we went to press in November, an FSIS spokesperson said that baseline data on the prevalence of campylobacter in broiler and turkey carcasses had been collected and were being analyzed and that draft performance standards based on those findings and a risk assessment would be ready by the year's end. FSIS testing for campylobacter would follow.

Carol L. Tucker-Foreman, distinguished fellow at the Consumer Federation of America's Food Policy Institute and a former USDA official, cited "at least a decade of promises and failures to develop campylobacter baseline data and a standard." But she acknowledged that the FSIS could deliver a report on baseline data by the end of 2009. "It is essential," she added, "to have a performance standard for campylobacter."

Behind the numbers

At 14 percent, the overall salmonella incidence is within the range we've seen in the past 12 years. In previous tests, the incidence ranged from 9 percent to 16 percent overall. Campylobacter incidence has varied more. Now it's 62 percent overall; in our previous tests it ranged from 42 percent to 81 percent.

When we took bacteria samples from contaminated chicken and analyzed their resistance to common antibiotics, most bugs could resist at least one antibiotic, and some evaded multiple classes of drugs. If a patient needs treatment, that might leave a doctor with poorer odds of choosing an effective antibiotic to fight infections that might be more stubborn.

The good news: All of the antibiotics were effective against 32 percent of the salmonella samples and 40 percent of the campylobacter samples. Back in January 2007, we reported that those figures were just 16 percent and 33 percent.

It's not surprising that we found antibiotic-resistant bacteria even in organic chickens, which are raised without antibiotics. "Chickens grown under organic conditions are given exposure to the outdoors, which provides contact with vermin such as rodents, insects, and birds that can carry and transmit these bacteria to chickens," said Michael Doyle, Ph.D., director of the University of Georgia's Center for Food Safety. Moreover, once genes for antibiotic resistance are in the gene pool of microbes, they can persist in the soil for years, even after the antibiotics are no longer in use.

The safeguards in place

Despite modest improvement in some numbers, our findings suggest that most companies' safeguards might be inadequate. To tease out what might account for Perdue's and Bell & Evans' relative success, we asked those companies as well as Tyson and Foster Farms whether they have added any food-safety measures in the past few years. We didn't reveal our test results.

Bruce Stewart-Brown, Perdue's vice president of food safety and quality, and a doctor of veterinary medicine, told us the company has increased its salmonella vaccinations over the past few years. That's designed to prevent chicks from picking up the bacterium from their mothers. Further protections, Stewart-Brown said, include an "all-in, all-out production model." Translation: Flocks are cleared out completely. Between flocks, farmers dry the empty chicken houses (which kills bacteria) and often use a product that temporarily changes the pH of the ground (to make it inhospitable to bacterial growth). Birds on each farm are the same age, so there are no older birds to contaminate newly arrived younger ones. "We also work closely with the farmers that raise our poultry," he said. "We make sure they isolate any other species of animals that might transfer microbiology to our chickens, use footwear and clothing control programs, and closely regulate visitation by outsiders."

Stewart-Brown also says that Perdue has implemented 25 food-safety steps at its processing plants.

Tom Stone, director of marketing at Bell & Evans, which produced those clean chickens, said the company has started packaging its products with a machine that seals the edges with film and shrinks the material, so there's no need for a "diaper" under the chicken to sop up fluids. "Our chickens are air-chilled and carry the 'No Retained Water' statement," he said.

But listen to Foster Farms and Tyson and you'd think they would have been as clean. Robert O'Connor, vice president of technical services at Foster Farms and a doctor of veterinary medicine, cited the company's use of "the most technologically advanced and proven systems available." Tyson spokesman Gary Mickelson said his company's safeguards include keeping hatcheries sanitized, vaccinating some breeder stock against salmonella, and ensuring proper refrigeration during product delivery.

Our own experts say that controlling the spread of bacteria is a matter of being vigilant and taking many small steps, from hatchery to store, rather than relying on one magic bullet. A May 2008 release of USDA compliance guidelines for the poultry industry recommends 37 "best practices," including controlling litter moisture in chicken houses and continuously rinsing carcasses and equipment in processing plants. Chicken producers that follow good practices in the hatchery and on the farm and abide by those government guidelines should be able to produce fewer chickens that harbor salmonella, though not necessarily campylobacter.

ConsumerReports.org®

How safe is that chicken?

Most tested broilers were contaminated

Last updated: January 2010

You would think that after years of alarms about food safety—outbreaks of illness followed by renewed efforts at cleanup—a staple like chicken would be a lot safer to eat. But in our latest analysis of fresh, whole broilers bought at stores nationwide, two-thirds harbored salmonella and/or campylobacter, the leading bacterial causes of foodborne disease. That's a modest improvement since January 2007, when we found that eight of 10 broilers harbored those pathogens. But the numbers are still far too high, especially for campylobacter. Though the government has been talking about regulating it for years, it has yet to do so. (See Viewpoint.)

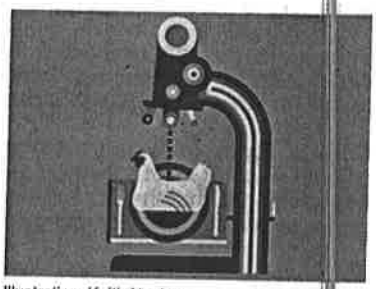


Illustration: Keith Negley

The message is clear: Consumers still can't let down their guard. They must cook chicken to at least 165° F and prevent raw chicken or its juices from touching any other food.
Illustration of chicken under microscope
Illustration by Keith Negley

Each year, salmonella and campylobacter from chicken and other food sources infect 3.4 million Americans, send 25,500 to hospitals, and kill about 500, according to estimates by the national Centers for Disease Control and Prevention. But the problem might be even more widespread: Many people who get sick don't seek medical care, and many of those who do aren't screened for foodborne infections, says Donna Rosenbaum, executive director of Safe Tables Our Priority, a national nonprofit food-safety organization. What's more, the CDC reports that in about 20 percent of salmonella cases and 55 percent of campylobacter cases, the bugs have proved resistant to at least one antibiotic. For that reason, victims who are sick enough to need antibiotics might have to try two or more before finding one that helps.

Consumer Reports has been measuring contamination in store-bought chickens since 1998. For our latest analysis, we had an outside lab test 382 chickens bought last spring from more than 100 supermarkets, gourmet- and natural-food stores, and mass merchandisers in 22 states. We tested three top brands—Foster Farms, Perdue, and Tyson—as well as 30 nonorganic store brands, nine organic store brands, and nine organic name brands. Five of the organic brands were labeled "air-chilled" (a slaughterhouse process in which carcasses are refrigerated and may be misted, rather than dunked in cold chlorinated water).

Among our findings:

- Campylobacter was in 62 percent of the chickens, salmonella was in 14 percent, and both bacteria were in 9 percent. Only 34 percent of the birds were clear of both pathogens. That's double the percentage of clean birds we found in our 2007 report but far less than the 51 percent in our 2003 report.
- Among the cleanest overall were air-chilled broilers. About 40 percent harbored one or both pathogens. Eight Bell & Evans organic broilers, which are air chilled, were free of both, but our sample was too small to determine that all Bell & Evans broilers would be.
- Store-brand organic chickens had no salmonella at all, showing that it's possible for chicken to arrive in stores without that bacterium riding along. But as our tests showed, banishing one bug doesn't mean banishing both: 57 percent of those birds harbored campylobacter.
- The cleanest name-brand chickens were Perdue's: 56 percent were free of both pathogens. This is the first time since we began testing chicken that one major brand has fared significantly better than others across the board.
- Most contaminated were Tyson and Foster Farms chickens. More than 80 percent tested positive for one or both pathogens.
- Among all brands and types of broilers tested, 68 percent of the salmonella and 60 percent of the campylobacter organisms we analyzed showed resistance to one or more antibiotics.

Dirty birds

As they're raised, chickens can peck at droppings and insects that carry salmonella and campylobacter. The bacteria settle in their intestines, usually without harm, and the chickens contaminate their environment with infected feces. When the birds are slaughtered, intestinal bacteria can wind up on their carcasses.

To minimize contamination, processors of poultry (and of meat and seafood) follow federally mandated procedures collectively known as HACCP (pronounced hass-ip), which stands for Hazard Analysis and Critical Control Point. Those measures are in effect in slaughterhouses and processing plants and are the consumer's main protection against contaminated chicken. HACCP, implemented for poultry and meat plants in 1997, requires companies to spell out where contamination might occur and then institute procedures to prevent, reduce, or eliminate it.

Inspectors for the U.S. Department of Agriculture's Food Safety and Inspection Service (FSIS) monitor chicken companies' HACCP plans. They inspect carcasses and viscera for tumors, bruises, and other defects. During testing periods, they also pluck a broiler a day off the line and test it for the presence of salmonella. Plants that produce more than 12

1996. Nationwide pork microbiological baseline data collection program: market hogs. April 1995–March 1996. Available at: http://www.fsis.usda.gov/OPHS/baseline/markhog1.pdf. Accessed 14 April 2006.

48. Vacher, S., A. Menard, E. Bernard, and F. Megraud. 2003. PCR-restriction fragment length polymorphism analysis for detection of point mutations associated with macrolide resistance in *Campylobacter* spp. *Antimicrob. Agents Chemother.* 47:1125–1128.
49. van de Giessen, A., S. I. Mazurier, W. Jacobs-Reitsma, W. Jansen, P. Berkers, W. Ritmeester, and K. Wernars. 1992. Study on the epidemiology and control of *Campylobacter jejuni* in poultry broiler flocks. *Appl. Environ. Microbiol.* 58:1913–1917.
50. Van Looveren, M., G. Daube, L. De Zutter, J. M. Dumont, C. Lammens, M. Wijdooghe, P. Vandamme, M. Jouret, M. Cornelis, and H. Goossens. 2001. Antimicrobial susceptibilities of *Campylobacter* strains isolated from food animals in Belgium. *J. Antimicrob. Chemother.* 48:235–240.
51. Wesley, I. V., S. J. Wells, K. M. Harmon, A. Green, L. Schroeder-Tucker, M. Glover, and I. Siddique. 2000. Fecal shedding of *Campylobacter* and *Arcobacter* spp. in dairy cattle. *Appl. Environ. Microbiol.* 66:1994–2000.
52. Yan, W., and D. E. Taylor. 1991. Characterization of erythromycin resistance in *Campylobacter jejuni* and *Campylobacter coli*. *Antimicrob. Agents Chemother.* 35:1989–1996.
53. Ziprin, R. L., C. R. Young, L. H. Stanker, M. E. Hume, and M. E. Konkel. 1999. The absence of cecal colonization of chicks by a mutant of *Campylobacter jejuni* not expressing bacterial fibronectin-binding protein. *Avian Dis.* 43:586–589.

T. S. Jones, E. A. Lockamy, C. M. Patton, and R. O. Sikes. 1987. *Campylobacter* enteritis at a university: transmission from eating chicken and from cats. *Am. J. Epidemiol.* 126:526–534.
11. Doyle, M. P., and D. M. Jones. 1992. Food-borne transmission and antibiotic resistance of *Campylobacter jejuni,* p. 45–48. *In* I. Nachamkin, M. J. Blaser, and L. S. Tompkins (ed.), *Campylobacter jejuni*: current status and future trends. ASM Press, Washington, D.C.
12. Engberg, J., F. M. Aarestrup, D. E. Taylor, P. Gerner-Smidt, and I. Nachamlin. 2001. Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli*: resistance mechanisms and trends in human isolates. *Emerg. Infect. Dis.* 7:24–34.
13. Englen, M. D., and P. J. Fedorka-Cray. 2002. Evaluation of a commercial diagnostic PCR for the identification of *Campylobacter jejuni* and *Campylobacter coli. Lett. Appl. Microbiol.* 35:353–356.
14. Garcia, M. M., H. Lior, R. B. Stewart, G. M. Ruckerbauer, J. R. Trudel, and A. Skljarevski. 1985. Isolation, characterization, and serotyping of *Campylobacter jejuni* and *Campylobacter coli* from slaughter cattle. *Appl. Environ. Microbiol.* 49:667–672.
15. Gibreel, A., V. N. Kos, M. Keelan, C. A. Trieber, S. Levesque, S. Michaud, and D. E. Taylor. 2005. Macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli*: molecular mechanisms and stability of the resistance phenotype. *Antimicrob. Agents Chemother.* 49:2753–2759.
16. Gupta, A., J. M. Nelson, T. J. Barrett, R. V. Tauxe, S. P. Rossiter, C. R. Friedman, K. W. Joyce, K. E. Smith, T. F. Jones, M. A. Hawkins, B. Shiferaw, J. L. Beebe, D. J. Vugia, T. Rabatsky-Ehr, J. A. Benson, T. P. Root, and F. J. Angulo. 2004. Antimicrobial resistance among *Campylobacter* strains, United States, 1997–2001. *Emerg. Infect. Dis.* 10:1102–1109.
17. Headrick, M. L., and L. Tollefson. 1998. Food borne disease summary by food commodity. *Vet. Clin. North Am. Food Anim. Pract.* 14:91–100.
18. Humphrey, T. J., A. Henley, and D. G. Lanning. 1993. The colonization of broiler chickens with *Campylobacter jejuni*—some epidemiological investigation. *Epidemiol. Infect.* 110:601–607.
19. Inglis, G. D., T. A. McAllister, H. W. Busz, L. J. Yanke, D. W. Morck, M. E. Olson, and R. R. Read. 2005. Effects of subtherapeutic administration of antimicrobial agents to beef cattle on the prevalence of antimicrobial resistance in *Campylobacter jejuni* and *Campylobacter hyointestinalis. Appl. Environ. Microbiol.* 71:3872–3881.
20. Ishihara, K., T. Kira, K. Ogikubo, A. Morioka, A. Kojima, M. Kijima-Tanaka, T. Takahashi, and Y. Tamura. 2004. Antimicrobial susceptibility of *Campylobacter* isolated from food-producing animals on farms (1999–2001): results from the Japanese Veterinary Antimicrobial Resistance Monitoring Program. *Int. J. Antimicrob. Agents* 24:63–69.
21. Jacobs-Reitsman, W. F. 1995. *Campylobacter* bacteria in breeder flocks. *Avian Dis.* 39:355–359.
22. Jacobs-Reitsman, W. F. 1997. Aspects of epidemiology of *Campylobacter* in poultry. *Vet. Q.* 19:113–117.
23. Jensen, L. B., and F. M. Aarestrup. 2001. Macrolide resistance in *Campylobacter coli* of animal origin in Denmark. *Antimicrob. Agents Chemother.* 45:371–372.
24. Jones, F. T., and S. C. Ricke. 2003. Observations on the history of the development of antimicrobials and their use in poultry feeds. *Poult. Sci.* 82:613–617.
25. Lastovica, A. J., and M. B. Skirrow. 2000. Clinical significance of *Campylobacter* and related species other than *Campylobacter jejuni* and *C. coli,* p. 89–120. *In* I. Nachamkin and M. J. Blaser (ed.), *Campylobacter,* 2nd ed. ASM Press, Washington, D.C.
26. Luber, P., J. Wagner, H. Hahn, and E. Bartelt. 2003. Antimicrobial resistance in *Campylobacter jejuni* and *Campylobacter coli* strains isolated in 1991 and 2001–2002 from poultry and humans in Berlin, Germany. *Antimicrob. Agents Chemother.* 47:3825–3830.
27. Manser, P. A., and R. W. Dalzeil. 1985. A survey of *Campylobacter* in animals. *J. Hyg.* 95:15–21.
28. McCrea, B. A., K. H. Tonooka, C. VanWorth, E. R. Atwill, and J. S. Schrader. 2006. Colonizing capability of *Campylobacter jejuni* genotypes from low-prevalence avian species in broiler chickens. *J. Food Prot.* 69:417–420.
29. McEwen, S. A., and P. J. Fedorka-Cray. 2002. Antimicrobial use and resistance in animals. *Clin. Infect. Dis.* 34(Suppl. 3):S93–S106.
30. McNulty, C. A. 1987. The treatment of *Campylobacter* infections in man. *J. Antimicrob. Chemother.* 19:281–284.
31. Meinersmann, R. J., L. O. Helsel, P. I. Fields, and K. L. Hiett. 1997. Discrimination of *Campylobacter jejuni* isolate by *fla* gene sequencing. *J. Clin. Microbiol.* 35:2810–2814.
32. Moore, J. E., and R. H. Madden. 1998. Occurrence of thermophilic *Campylobacter* spp. in porcine liver in Northern Ireland. *J. Food Prot.* 61:409–413.
33. Nachamkin, I., J. Engberg, and F. M. Aarestrup. 2000. Diagnosis and antimicrobial susceptibility of *Campylobacter* spp., p. 45–66. *In* I. Nachamkin and M. J. Blaser (ed.), *Campylobacter,* 2nd ed. ASM Press, Washington D.C.
34. NCCLS. 2002. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Approved standard, 2nd ed. NCCLS document M31-A2. NCCLS, Wayne, Pa.
35. Payot, S., L. Avrain, C. Magras, K. Praud, A. Cloeckaert, and E. Chaslus-Dancla. 2004. Relative contribution of target gene mutation and efflux to fluoroquinolone and erythromycin resistance, in French poultry and pig isolates of *Campylobacter coli. Int. J. Antimicrob. Agents* 23:468–472.
36. Pearce, R., R. Dudley, F. M. Wallace, J. E. Call, and J. B. Luchansky. 2002. Prevalence and distribution of *Campylobacter* spp. in a swine slaughter and processing facility. *J. Anim. Sci.* 80(Suppl. 1):262. (Abstract.)
37. Pearson, A. D., M. H. Greenwood, R. K. A. Feltham, T. D. Healing, J. Donaldson, D. M. Jones, and R. R. Colwell. 1996. Microbial ecology of *Campylobacter jejuni* in a United Kingdom chicken supply chain: intermittent common source, vertical transmission, and amplification by flock propagation. *Appl. Environ. Microbiol.* 62:4614–4620.
38. Purdy, D., S. Cawthraw, J. H. Dickinson, D. G. Newell, and S. F. Park. 1999. Generation of a superoxide dismutase (SOD)-deficient mutant of *Campylobacter coli*: evidence for the significance of SOD in *Campylobacter* survival and colonization. *Appl. Environ. Microbiol.* 65:2540–2546.
39. Sáenz, Y., M. Zarazaga, M. Lantero, M. J. Gastanares, F. Baquero, and C. Torres. 2000. Antibiotic resistance in *Campylobacter* strains isolated from animals, foods, and humans in Spain in 1997–1998. *Antimicrob. Agents Chemother.* 44:267–271.
40. Samuel, M. C., D. J. Vugia, S. Shallow, R. Marcus, S. Segler, T. McGivern, H. Kassenborg, K. Reilly, M. Kennedy, F. Angulo, and R. V. Tauxe. 2004. Epidemiology of sporadic *Campylobacter* infection in the United States and declining trend in incidence, FoodNet 1996–1999. *Clin. Infect. Dis.* 38(Suppl. 3):S165–S174.
41. Stanley, K. N., J. S. Wallace, J. E. Currie, P. J. Diggle, and K. Jones. 1998. The seasonal variation of thermophilic campylobacters in beef cattle, dairy cattle, and calves. *J. Appl. Microbiol.* 85:472–480.
42. Stas, T., F. T. W. Jordon, and Z. Woldehiwet. 1999. Experimental infection of chickens with *Campylobacter jejuni:* strains differ in their capacity to colonize the intestine. *Avian Pathol.* 28:61–64.
43. Stern, N. J. 1992. Reservoirs for *Campylobacter jejuni* and approaches for intervention in poultry, p. 49–60. *In* I. Nachamkin, M. J. Blaser, and L. S. Tompkins (ed.), *Campylobacter jejuni*: current status and future trends. ASM Press, Washington, D.C.
44. Stern, N. J., P. Fedorka-Cray, J. S. Bailey, N. A. Cox, S. E. Craven, K. L. Hiett, M. T. Musgrove, S. Ladely, and D. Cosby. 2001. Distribution of *Campylobacter* spp. in selected U.S. poultry production and processing operations. *J. Food Prot.* 64:1705–1710.
45. Stern, N. J., B. Wojton, and K. Kwiatek. 1992. A differential-selective medium and dry ice-generated atmosphere for recovery of *Campylobacter jejuni. J. Food Prot.* 55:514–517.
46. Trieber, C. A., and D. E. Taylor. 2000. Mechanisms of antibiotic resistance in *Campylobacter,* p. 441–454. *In* I. Nachamkin and M. J. Blaser (ed.), *Campylobacter.* ASM Press, Washington, D.C.
47. U.S. Department of Agriculture, Food Safety Inspection Service.

TABLE 5. *Distribution of erythromycin MICs for* C. jejuni *and* C. coli *recovered from ceca samples at 3, 4, 5, and 6 weeks of age from broilers receiving subtherapeutic (22-ppm) or therapeutic (529-ppm) concentrations of tylosin*

Species and concn of tylosin	Age (wk)	No. of isolates with indicated MIC (μg/ml):								
		1	2	4	8[a]	16	32	64	128	>256
C. jejuni										
22 ppm	3	10	2	—	—	—	—	—	—	—
	4	3	4	1	—	—	—	—	—	7
	5	1	—	—	2	—	—	—	—	12
	6	—	—	4	—	—	—	—	1	10
529 ppm	3	4	—	—	—	—	—	—	—	—
	4	10	—	—	—	—	—	—	—	—
	5	10	—	1	—	—	—	—	1	—
	6	7	3	—	—	—	—	—	—	2
C. coli										
22 ppm	3	1	—	—	—	—	—	—	—	—
	4	—	—	2	—	—	—	—	—	—
	5	—	—	—	—	—	—	—	—	5
	6	—	—	—	—	—	—	—	—	10
529 ppm	3	—	—	—	—	—	—	—	—	—
	4	—	—	—	—	—	—	—	—	—
	5	3	—	—	—	—	—	—	—	—
	6	1	—	—	—	—	—	—	—	2

[a] Erythromycin MICs ≥8 μg/ml were considered resistant *(34)*.

acquisition of resistance has led to speculation that resistance in *Campylobacter* differs, depending on both the species and host animal *(20)*.

Limited data are available regarding the development of resistance from the subtherapeutic administration of antimicrobials in livestock production. Inglis et al. *(19)* reported that the administration of tylosin at subtherapeutic concentrations did not influence the carriage rates of erythromycin-resistant *Campylobacter* in feedlot cattle. The prevalence of erythromycin resistance observed in *C. coli* from broilers administered tylosin at subtherapeutic concentrations is similar to that commonly observed in swine isolates *(2, 20, 39)*. Although the relatively low prevalence of macrolide-resistant *Campylobacter* in broilers typically reported in survey data compared with swine isolates may reflect substantially less macrolide use in poultry production, this is difficult to establish. In the United States, published estimates of antimicrobial use in food animal production differ markedly *(24)*. Nevertheless, our findings are consistent with the majority of data indicating that a significantly higher proportion of *C. coli* isolates are macrolide resistant than are *C. jejuni,* regardless of the source of isolates.

In addition, we observed significantly higher frequencies of macrolide resistance when tylosin was administered at subtherapeutic compared with therapeutic concentrations (Table 5). The administration of tylosin at therapeutic concentrations significantly reduced cecal *Campylobacter* counts (Tables 3 and 4) and the prevalence of *Campylobacter* (Table 2). These trends were less obvious when tylosin was administered at subtherapeutic concentrations, in which case, the dose administered in combination with the rate of feed consumption appeared to be inadequate in inhibiting *Campylobacter* growth, with an end result of emerging resistance under selective pressure. Further studies are needed to determine the factors involved in the apparent difference in the acquisition of macrolide resistance in *C. coli* compared with *C. jejuni.*

REFERENCES

1. Aarestrup, F. M., and J. Engberg. 2001. Antimicrobial resistance of thermophilic *Campylobacter*. *Vet. Res.* 32:311–321.
2. Aarestrup, F. M., M. Nielsen, M. Madsen, and J. Engberg. 1997. Antimicrobial susceptibility patterns of thermophilic *Campylobacter* spp. from humans, cattle, and broilers in Denmark. *Antimicrob. Agents Chemother.* 41:2244–2250.
3. Aarestrup, F. M., and H. C. Wegner. 1999. The effects of antibiotic usage in food animals on the development of antimicrobial resistance of importance for humans in *Campylobacter* and *Escherichia coli*. *Microbes Infect.* 1:639–644.
4. Ahmed, I. H., G. Manning, T. M. Wassenaar, S. Cawthraw, and N. G. Newell. 2002. Identification of genetic differences between two *Campylobacter jejuni* strains with different colonization potentials. *Microbiology* 148:1203–1212.
5. Allos, B. M. 2001. *Campylobacter jejuni* infections: update on emerging issues and trends. *Clin. Infect. Dis.* 32:1201–1206.
6. Atabay, H. I., and J. E. Corry. 1998. The isolation and prevalence of campylobacters from dairy cattle using a variety of methods. *J. Appl. Microbiol.* 84:733–740.
7. Bras, A. M., S. Chatterjee, B. W. Wren, D. G. Newell, and J. M. Ketley. 1999. A novel *Campylobacter jejuni* two-component regulatory system important for temperature-dependent growth and colonization. *J. Bacteriol.* 81:3298–3302.
8. Compendium of Veterinary Products. 2003. Compendium of veterinary products. North American Compendiums, Inc., Port Huron, Mich.
9. Corcoran, D., T. Quinn, L. Cotter, and S. Fanning. 2006. An investigation of the molecular mechanisms contributing to high-level erythromycin resistance in *Campylobacter*. *Int. J. Antimicrob. Agents* 27:40–45.
10. Deming, M. S., R. V. Tauxe, P. A. Blake, S. E. Dixion, B. S. Fowler,

TABLE 4. *Mean counts of* Campylobacter coli *in ceca samples at 2, 3, 4, 5, and 6 weeks of age from broilers receiving no medication (0), subtherapeutic (22-ppm) concentration, or therapeutic (529-ppm) concentration of tylosin*

Age (wk)	Log CFU/g (SE)		
	0	22 ppm[a]	529 ppm
2	1.14 A[b] (0.63)	5.60 A (1.06)	5.42 A (1.04)
3	0.44 A (0.44)	0.25 B (0.25)	0 B
4	2.90 AB (1.10)	0.89 B (0.62)	0 B
5	5.82 B (1.11)	2.89 AB (1.09)	1.09 B (0.59)
6	5.05 B (0.98)	5.10 A (1.02)	1.37 B (0.76)

[a] Medication treatments were initiated after sample collection at 2 weeks of age.

[b] Values with different letters within a column are significantly different ($P < 0.05$) by Tukey's honest significant difference.

of therapeutic concentration treatment and remained significantly lower than nonmedicated broilers and those receiving tylosin at a subtherapeutic concentration.

In contrast to broilers exposed to *C. jejuni,* the prevalence of *C. coli* differed significantly across treatment groups prior to the administration of medication treatments. Initial low *C. coli* colonization in the nonmedicated control group confounds comparisons across groups at a given age. However, analysis within a treatment group by age indicates that *C. coli* counts in ceca plus contents were also reduced following the administration of tylosin (Table 4). For broilers receiving tylosin at therapeutic concentrations (529 ppm), *C. coli* counts were reduced significantly ($P < 0.05$) 1 week after the administration of treatment and remained lower for the remainder of the study compared with counts from the same treatment group prior to the administration of medication. For broilers receiving tylosin at subtherapeutic concentrations (22 ppm), *C. coli* counts were reduced significantly ($P < 0.05$) 1 week after the administration of treatment and remained lower ($P < 0.05$) until week 5 compared with counts in the same group prior to the administration of medication.

Over the course of the study, the MIC of erythromycin was determined for 325 *Campylobacter* isolates (237 *C. jejuni* and 88 *C. coli*). No macrolide-resistant strains were recovered from broilers that had not been administered tylosin. However, macrolide-resistant *C. jejuni* and *C. coli* strains were recovered from broilers administered tylosin at both subtherapeutic and therapeutic concentrations. Erythromycin-resistant *C. jejuni* strains were recovered 2 weeks after the administration of subtherapeutic concentrations of tylosin (4 weeks of age) and 3 weeks after the administration of therapeutic concentrations (5 weeks of age). Resistant *C. coli* isolates were recovered 3 and 4 weeks after the administration of medication for subtherapeutic and therapeutic treatments, respectively. The SVR of the *flaA* gene was sequenced for 38 erythromycin-resistant *Campylobacter* isolates (26 *C. jejuni* and 12 *C. coli*) recovered from broiler ceca following the administration of tylosin in either feed or drinking water. Cluster analysis of the *flaA* SVR sequences showed that macrolide resistance was induced in each of the strains that colonized broilers; however, the recovery of resistant strains varied across replications (Table 1). Induction of resistance to macrolides occurs as a result of mutation of the 23S rRNA gene. Mutations, as stochastic events, can be expected to vary between trials.

The distribution of erythromycin MICs for *C. jejuni* and *C. coli* recovered after the initiation of medication treatments are shown in Table 5. Erythromycin resistance was observed at a higher frequency ($P < 0.01$) among *C. coli* isolates (70.8% [17 of 24]) than among *C. jejuni* isolates (36.8% [35 of 95]). In addition, erythromycin resistance was observed at a significantly higher frequency ($P < 0.001$) when tylosin was administered at subtherapeutic concentrations (62.7% [47 of 75]) than at therapeutic concentrations (11.4% [5 of 44]). Eighty-three percent (15 of 18) of the *C. coli* isolates recovered following the administration of tylosin at subtherapeutic concentrations were resistant to erythromycin compared with only 33.3% (2 of 6) following the administration of therapeutic concentrations. The erythromycin MIC_{50}s for *C. coli* isolates recovered following the subtherapeutic and therapeutic administration of tylosin were >256 and 1 μg/ml, respectively. Similarly, 56.1% (32 of 57) of the *C. jejuni* isolates recovered following the administration of tylosin at subtherapeutic concentrations were resistant to erythromycin (MIC_{50}, 128 μg/ml) compared with 7.9% (3 of 38) of the *C. jejuni* isolates recovered following the administration therapeutic concentrations of tylosin (MIC_{50}, 1 μg/ml).

DISCUSSION

The *C. jejuni* strains used in this study were substantially better colonizers of broilers than the *C. coli* stains. Differences in the colonizing potential of *Campylobacter* strains in broilers have been previously reported *(4, 28, 42),* and several genes, including *sodB, racR,* and *cadF (7, 38, 53),* have been identified as colonization factors. Genetic variation in the isolates used or host factors may have contributed to the lower prevalence of *Campylobacter* observed in broilers exposed to *C. coli* strains in our study.

We observed erythromycin resistance at a higher prevalence among *C. coli* than among *C. jejuni* broiler isolates. Antimicrobial resistance surveys from Belgium, Denmark, and Japan have reported analogous findings, with 18 to 35% of *C. coli* isolates recovered from broilers expressing macrolide resistance compared with only 0 to 6% of *C. jejuni* isolates *(2, 20, 50).* However, Sáenz et al. *(39)* reported no macrolide-resistant *C. coli* or *C. jejuni* among broiler isolates in Spain. Prevalence data similar to ours have also been observed among human isolates where 8 to 34% of *C. coli* and 0 to 3% of *C. jejuni* were reported to be erythromycin resistant *(2, 16, 26, 39).* Survey data indicate that the prevalence of macrolide resistance among swine *Campylobacter* isolates tends to be two- to threefold higher than among strains isolated from other food animals or humans, with resistance among *C. coli* ranging from 48 to 81% and among *C. jejuni* from 0 to 33% *(2, 20, 39, 50).* *C. jejuni* is most commonly associated with poultry and human campylobacterosis, while *C. coli* is primarily associated with swine. This host specificity and difference in

TABLE 1. Campylobacter *strain recovery and development of erythromycin (ERY) resistance in each experimental replications (reps)*

Species and isolate	Strain recovery			ERY-resistant strains recovered		
	Reps			Reps		
	1	2	3	1	2	3
C. jejuni 3936	+	+	+	+	+	+
C. jejuni 4820	+	+	+	+	+	−
C. jejuni 39364	−	−	−	−	−	−
C. coli 6647	+	+	+	−	+	−
C. coli 97756	−	−	−	−	−	−
C. coli 98544	+	+	+	+	+	+

Statistical analysis. *Campylobacter* counts were log transformed and analyzed by the general linear model (Statistica, StatSoft, Tulsa, Okla.). Count means were separated by Tukey's honest significant difference. The χ^2 test was used for evaluating differences in prevalence and differences in macrolide resistance frequencies between *C. jejuni* and *C. coli.*

RESULTS

Throughout the study, no *Campylobacter* was recovered from broilers that had not been experimentally exposed to *Campylobacter. C. jejuni* was the only *Campylobacter* species recovered from birds exposed to *C. jejuni,* and *C. coli* was the only species recovered from birds exposed to *C. coli,* as determined by BAX PCR. The SVR of the *flaA* gene was sequenced for 46 macrolide-susceptible *Campylobacter* isolates (28 *C. jejuni* and 18 *C. coli*) recovered from broiler ceca at 2 weeks of age, prior to the administration of medication treatments. Sequence comparisons with *flaA* SVR reference sequences prepared from each of the inoculum strains showed that two of the three strains utilized in each of the challenge cocktails (*C. jejuni* and *C. coli*) were recovered from broiler ceca (Table 1). These findings were consistent across replications.

A higher prevalence of *Campylobacter* detection was observed among broilers exposed to *C. jejuni* than among broilers exposed to *C. coli* (Table 2). At 2 weeks of age, prior to the administration of medication treatments, 75.6% (68 of 90) of all birds exposed to *Campylobacter* were detected positive, 100% (45 of 45) of those exposed to *C. jejuni* were found positive, while only 51.1% (23 of 45) of those exposed to *C. coli* were found positive. *Campylobacter* prevalence rates remained at 100% (15 of 15) across sampling dates in nonmedicated broilers exposed to *C. jejuni,* whereas the prevalence rate in nonmedicated broilers exposed to *C. coli* was only 20% (3 of 15) at 2 weeks of age and never exceeded 66.7% (10 of 15) throughout the study.

By 3 weeks of age (1 week posttreatment), the prevalence of *Campylobacter*-positive broilers (Table 2) and the levels of *Campylobacter* observed in ceca plus contents (Tables 3 and 4) were inversely related to the administration of tylosin. Total *Campylobacter* counts from CCA and eCCA plates did not differ significantly for broilers administered tylosin (data not shown). *Campylobacter* counts (presented in Tables 3 and 4) for nonmedicated broilers and medicated broilers prior to medication treatment were from CCA plates. *Campylobacter* counts for medicated broilers, following the administration of medication treatments (weeks 3 to 6), were from eCCA plates. Among broilers exposed to *C. jejuni, Campylobacter* prevalence and cecal counts (CFU per gram of ceca plus contents) were significantly reduced ($P < 0.05$) 1 week after the administration

TABLE 3. *Mean counts of* Campylobacter jejuni *in ceca samples at 2, 3, 4, 5, and 6 weeks of age from broilers receiving no medication (0), subtherapeutic (22-ppm) concentration, or therapeutic (529-ppm) concentration of tylosin*

Age (wk)	Log CFU/g (SE)		
	0	22 ppm[a]	529 ppm
2	7.09 A[b] (0.32)	7.67 A (0.16)	7.55 A (0.29)
3	7.02 A (0.18)	4.68 B (0.72)	1.75 C (0.78)
4	7.87 A (0.20)	7.22 A (0.35)	4.28 B (0.84)
5	8.04 A (0.18)	7.77 A (0.18)	5.08 B (0.71)
6	7.70 A (0.11)	7.71 A (0.14)	5.37 B (0.73)

[a] Medication treatments were initiated after sample collection at 2 weeks of age.

[b] Values with different letters within a row are significantly different ($P < 0.05$) by Tukey's honest significant difference.

TABLE 2. *Prevalence of* C. jejuni– *and* C. coli–*positive birds at 2, 3, 4, 5, and 6 weeks of age in broilers receiving no medication (0), subtherapeutic (22-ppm) concentrations, or therapeutic (529-ppm) concentrations of tylosin*

Age (wk)	No. (%) positive by week[a]							
	C. jejuni				*C. coli*			
	0	22 ppm	529 ppm	*P* value[b]	0	22 ppm	529 ppm	*P* value
2	15 (100)	15 (100)	15 (100)	1	3 (20)	10 (67)	10 (67)	0.013
3	15 (100)	12 (80)	4 (27)	<0.001	1 (7)	1 (7)	0	0.593
4	15 (100)	15 (100)	10 (67)	0.004	5 (33)	2 (13)	0	0.041
5	15 (100)	15 (100)	12 (80)	0.041	10 (67)	5 (33)	3 (20)	0.028
6	15 (100)	15 (100)	12 (80)	0.041	10 (67)	10 (67)	3 (20)	0.013

[a] Data combined from three replications; 5 broilers were sampled weekly in each replication for a total of 25 broilers for each replication.

[b] Within species and age group, the probability that differences existed in the group was calculated with the chi-square test for independence.

(12, 23, 46, 48). Efflux systems have also been shown to provide low-level macrolide resistance in *Campylobacter (9, 35).* Other mechanisms conferring macrolide resistance, such as mutations in ribosomal proteins, methylation of the drug binding site, and drug inactivation, have not yet been observed in *Campylobacter (15, 52).*

The objective of this study was to evaluate the effect of administering therapeutic and subtherapeutic concentrations of tylosin on the erythromycin susceptibility of *C. jejuni* and *C. coli* isolated from the ceca of treated broilers.

MATERIALS AND METHODS

Study design. In each of three replicate studies, 175 day-of-hatch chicks were obtained from a commercial broiler hatchery and were allotted to one of seven groups of 25 birds each. Treatment groups were placed in separate isolation rooms on pine shavings and were provided a standard nonmedicated broiler starter-grower diet and water ad libitum. All procedures were administered in accordance to protocols approved by an institutional animal care and use committee.

At placement, three groups of 25 birds each were exposed to *C. jejuni* by commingling with two seeder chicks that had been challenged by oral gavage with 10^7 CFU of a cocktail of three strains of macrolide-susceptible *C. jejuni.* In a similar manner, three groups were exposed to three strains of macrolide-susceptible *C. coli.* Seeder birds were marked at challenge and were not included in sample collection. A control group was not exposed to *Campylobacter* to determine the *Campylobacter* colonization status of chicks from the hatchery.

At 2 weeks of age, tylosin phosphate (Tylan 10, Elanco Animal Health, Indianapolis, Ind.) was administered in the diet at a subtherapeutic concentration of 22 ppm (20 g of active ingredient per ton; U.S. Food and Drug Administration–approved concentration for increased rate of weight gain and improved feed efficiency in broilers) to one group of birds exposed to *C. jejuni* and one group exposed to *C. coli.* Tylosin-medicated feed was provided ad libitum to these two groups for the remainder of the study (4 weeks). At this same time, tylosin tartrate (Tylan Soluble, Elanco Animal Health) was administered at a therapeutic concentration of 529 ppm (0.5291 g/liter; U.S. Food and Drug Administration–approved concentration for the treatment of chronic respiratory disease in broilers) in the drinking water for 5 days to one group of birds exposed to *C. jejuni* and one group exposed to *C. coli.* Medications were administered in accordance with the manufacturer's label directions. Three control groups, one exposed to *C. jejuni,* one exposed to *C. coli,* and one unexposed group, were not administered tylosin in either the feed or drinking water.

***Campylobacter* strains and inoculum preparation.** All strains used in these studies were obtained from the animal arm of the National Antimicrobial Resistance Monitoring System *Campylobacter* collection located at the U.S. Department of Agriculture, Agricultural Research Service, Russell Research Center in Athens, Ga. Strains were originally isolated from poultry carcass rinses. Previous testing had determined that the isolates were susceptible to azithromycin and erythromycin. In addition, they were susceptible to ciprofloxacin, clindamycin, chloramphenicol, gentamicin, nalidixic acid, and tetracycline. Cocktails were prepared that consisted of three strains of *C. jejuni* (3936, 4820, and 39364) or three strains of *C. coli* (6647, 97756, and 98544). Challenge cultures were prepared by inoculating frozen stock cultures of each strain onto blood agar (tryptic soy agar with 5% sheep blood; Difco, Becton Dickinson, Sparks, Md.) and incubating at 42°C for 24 h in a sealable bag flushed with a microaerobic gas mixture (5% O_2, 10% CO_2, and 85% N_2). Freshly grown colonies of each of the three strains were suspended as a mixture in phosphate-buffered saline (PBS, 0.9%, pH 7.2) and adjusted to a final concentration of 10^8 CFU/ml with an A_{540} of 0.45 (Spectronic 20, Spectronics Instruments Inc., Rochester, N.Y.). Inoculum levels were confirmed by spread plating serial dilutions of each inoculum in duplicate.

***Campylobacter* recovery and identification.** At 2 weeks of age, prior to medication treatments, and at 3, 4, 5, and 6 weeks of age, five broilers per group were necropsied. Ceca were removed aseptically, placed in Whirl-Pak bags (Nasco, Modesto, Calif.) on ice, and processed within 2 h. Total and resistant *Campylobacter* spp. were enumerated from individual ceca plus contents as described below.

For the enumeration of total and macrolide-resistant *Campylobacter,* individual ceca were crushed to expose the contents, diluted 1:3 (wt/vol) with sterile PBS, and mixed in a stomacher (Seward Ltd., London, UK) for 30 s. Serial dilutions were prepared and plated onto duplicate Campy-Cefex agar (CCA) *(45)* and Campy-Cefex agar supplemented with erythromycin (eCCA) at 8 μg/ml. All plates were incubated at 42°C for 36 to 48 h microaerobically. Total and resistant populations of *Campylobacter* were estimated by plate counts on CCA and eCCA and are reported as log CFU per gram of ceca plus contents.

One presumptive *Campylobacter* colony was selected from CCA and one from eCCA for each sample for species identification and susceptibility testing. Presumptive identification consisted of observation of cellular morphology and motility by phase-contrast microscopy. Isolates were identified by a commercial multiplex PCR specific for *C. jejuni* and *C. coli* (BAX PCR, DuPont Qualicon, Wilmington, Del.), as previously described *(13).*

To determine which *Campylobacter* strains colonized broilers and which strains developed macrolide resistance, reference sequences of the short variable region (SVR) of the *flaA* gene of each of the inoculum strains were prepared and compared with *flaA* SVR sequences of subsets of macrolide susceptible and resistant isolates recovered throughout the study. The SVR was amplified as previously described with *flaA*-specific primers *(31).* Sequencing reactions were performed with the BigDye Terminator 1.1 Cycle Sequencing kit (Applied Biosystems, Foster City, Calif.) and an ABI 3100 Genetic Analyzer (Applied Biosystems) according to manufacturer's directions. Forward and reverse sequence data were assembled and compared by Sequencher version 4.2 (Gene Codes Corporation, Ann Arbor, Mich.).

Antimicrobial susceptibility testing. The MICs of erythromycin for all *Campylobacter* isolates recovered from individual ceca plus contents were determined by the agar dilution method recommended by the Clinical and Laboratory Standards Institute (formerly the NCCLS) *(34).* Erythromycin is recommended for macrolide susceptibility testing of *Campylobacter,* as interpretive standards for tylosin susceptibility testing have not been established. Nine doubling concentrations of erythromycin (Sigma, St. Louis, Mo.) were tested (range, 1 to 256 μg/ml) with Mueller-Hinton agar containing 5% defibrinated sheep blood. Isolates were tested on duplicate plates incubated at 42°C for 24 h under microaerobic conditions and were considered resistant to erythromycin if the MIC was ≥8 μg/ml. *C. jejuni* ATCC 33560 was used as a quality control strain, and its erythromycin MIC remained at 1 μg/ml throughout the study, which falls within the Clinical and Laboratory Standards Institute recommended range (1 to 4 μg/ml) under the growth conditions described *(34).*

Journal of Food Protection, Vol. 70, No. 8, 2007, Pages 1945–1951

Research Note

Development of Macrolide-Resistant *Campylobacter* in Broilers Administered Subtherapeutic or Therapeutic Concentrations of Tylosin†

SCOTT R. LADELY,[1] MARK A. HARRISON,[2] PAULA J. FEDORKA-CRAY,[1] MARK E. BERRANG,[1] MARK D. ENGLEN,[1] AND RICHARD J. MEINERSMANN[1]*

[1]*U.S. Department of Agriculture, Agricultural Research Service, Bacterial Epidemiology and Antimicrobial Resistance Research Unit, Richard B. Russell Agricultural Research Center, 950 College Station Road, Athens, Georgia 30605-2720; and* [2]*Department of Food Science and Technology, University of Georgia, Athens, Georgia 30602, USA*

MS 07-098: Received 21 February 2007/Accepted 20 April 2007

ABSTRACT

The use of antimicrobials in food animal production, particularly those commonly used to treat infections in humans, has become a source of debate in recent years. However, limited data are available regarding the development of resistance following the subtherapeutic or therapeutic administration of antimicrobials in animal production. The objective of this study was to evaluate the effect of the administration of therapeutic and subtherapeutic concentrations of tylosin on the erythromycin susceptibility of *Campylobacter jejuni* and *Campylobacter coli* isolated from the ceca of treated broilers. In three replicated studies, day-of-hatch chicks were exposed to macrolide-susceptible *C. jejuni* or *C. coli*. At 2 weeks of age, tylosin was administered at subtherapeutic (22 ppm, continuously in the diet) or therapeutic concentrations (529 ppm, in the drinking water for 5 days). Broilers were sacrificed weekly. Total and erythromycin-resistant *Campylobacter* spp. were enumerated from individual ceca plus cecal contents. Overall erythromycin resistance was observed at a higher frequency ($P < 0.01$) among *C. coli* isolates (70.8%) than among *C. jejuni* isolates (36.8%) following tylosin administration. Across *Campylobacter* species, erythromycin resistance was observed at a higher frequency ($P < 0.001$) when tylosin was administered at subtherapeutic (62.7%) than at therapeutic (11.4%) concentrations. Subtherapeutic administration resulted in the recovery of 83.3 and 56.1% erythromycin-resistant isolates compared with only 33.3 and 7.9% of the isolates expressing erythromycin resistance following the administration of therapeutic concentrations for *C. coli* and *C. jejuni*, respectively. Further studies are needed to determine the factors involved in the apparent difference in the acquisition of macrolide resistance in *C. coli* compared with *C. jejuni*.

Campylobacter spp. are estimated to account for 1.5 million cases of human gastroenteritis every year in the United States *(40)*. *Campylobacter jejuni* and *Campylobacter coli* are the species most frequently isolated from cases of human infection, with *C. jejuni* accounting for over 90% of infections and *C. coli* being identified in most of the remaining cases *(25, 30)*. Most cases of gastroenteritis result in a self-limiting diarrheal disease that does not require antimicrobial therapy. However, prolonged duration of illness, or altered immune function in some individuals, may warrant antimicrobial therapy *(1, 5)*.

A number of studies have investigated the epidemiology of *Campylobacter* in poultry production *(18, 21, 22, 37, 44, 49)*, because poultry products are considered a significant source of *Campylobacter* infections in humans *(10, 11)*. Prevalence studies in swine *(26)* and cattle *(6, 14, 27, 41, 51)* indicate that *Campylobacter* is a common commensal in other livestock production systems as well. Accordingly, pork, beef, and dairy products can also be a source of human *Campylobacter* infections *(17, 32, 36, 43, 47)*.

Considerable debate surrounds the use of antimicrobials in food animal production, especially those commonly used to treat human infections. Concerns regarding the emergence of resistant bacterial pathogens resulting from the use of antimicrobials in animals and the potential transfer of resistant strains from food products to humans have led to changes in antimicrobial use in food animal production worldwide *(3, 29)*. In U.S. food animal production, macrolides (erythromycin, tilmicosin, and tylosin) can be used to treat and prevent disease in poultry, swine, and beef cattle *(8)*. In addition, tylosin and oleandomycin are approved for use in poultry and swine diets for increased rate of weight gain and improved feed efficiency *(8)*. Erythromycin also serves as a primary treatment option for campylobacterosis in humans *(33)*. Even though the four aforementioned compounds have slight structural differences, their mode of action is the same; discrete point mutations in the 23S rRNA gene of *Campylobacter* confer cross-resistance among all members of the macrolide class of drugs

* Author for correspondence. Tel: 706-546-3236; Fax: 706-546-3066; E-mail: rick.meinersmann@ars.usda.gov.

† Mention of trade names or commercial products in this manuscript is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

near swine farms may be a potential environmental reservoir for both antimicrobial resistant bacteria and antimicrobial resistance genes. It is clear that swine farms are negatively impacting the shallow groundwater in eastern North Carolina. The extent to which such contamination of groundwater with multiple antibiotic-resistant *E. coli* poses risks to human health is uncertain and deserves further investigation.

References

Anon (1987). Drinking water microbiology, Committee on the Challenges of Modern Society (NATO/CCMS). *J. Environ. Pathol. Toxicol. Oncol.*, **7**, 1–365.

APHA (1998). *Standard Methods for the Examination of Water and Wastewater*, 20th edn, APHA, AWWA, WEF, Washington, DC.

CDC (2002). Surveillance summaries: surveillance for waterborne disease outbreaks–United States 1999–2000. *MMWR*, **51**(SS–8).

Edberg, S.C., LeClerc, H. and Robertson, J. (1997). Natural protection of spring and well drinking water against surface microbial contamination. II. Indicators and monitoring parameters for parasites. *Crit. Rev. Microbiol.*, **23**, 179–206.

Fanuel, M. (1999). *Modeling of Groundwater Flow Through Riparian Buffers* Masters Thesis, Dept. of Marine, Earth and Atmospheric Sciences, North Carolina State University, Raleigh N.C.

Marshall, B., Petrowski, D. and Levy, S.B. (1990). Inter- and intra- species spread of *Escherichia coli* in a farm environment in the absence of antibiotic usage. *Proc. Nat. Acad. Sci. USA*, **87**, 6609–6613.

McDermott, P.F., Zhao, S., Wagner, D.D., Simjee, S., Walker, R.D. and White, D.G. (2002). The food safety perspective of antibiotic resistance. *Anim. Biotechnol.*, **13**, 71–84.

Purdue Research Foundation (1996). Chemotherapy – Drug Groups. Cited August 19, 2002, http://www.vet.purdue.edu/depts/bms/courses/bms514/chmrx/chmrxtit.htm#Protein%20Synthesis%20Inhibitors.

Spruill, T.B. (2000). Statistical evaluation of effects of riparian buffers on nitrate and ground water quality. *J. Env. Qual.*, **29**, 1523–1538.

Stone, K.C., Hunt, P.G., Humenik, F.J. and Johnson, M.H. (1998). Impact of swine waste application on ground and stream water quality in an eastern coastal plain watershed. *Trans. ASAE*, **41**, 1665–1670.

Teale, C.J. (2002). Antimicrobial resistance and the food chain. *J. Appl. Microbiol.*, **92**(Suppl), 85S–89S.

Tollefson, L., Fedorka-Cray, P.J. and Angulo, F.J. (1999). Public health aspects of antibiotic resistance monitoring in the USA. *Acta Vet. Scand. Suppl.*, **92**, 67–75.

White, D.G., Hudson, C.R., Maurer, J.J., Ayers, S., Zhao, S., Lee, M.D., Bolton, L., Foley, T. and Sherwood, J. (2001). Characterisation of chloramphenicol and florfenicol resistance in *Escherichia coli* associated with bovine diarrhoea. Cited 3/9/2003: http://www.nal.usda.gov/ttic/tektran/data/000012/11/0000121170.html, Agricultural Research Service.

WHO (2004). *Guidelines for Drinking-water Quality: Vol 1 – Recommendations*, 3rd edn, World Health Organization, Geneva.

trimethoprim which might explain the presence of this resistance trait in bacteria associated with this swine farm. One in ten *E. coli* isolates found at reference site #1 was resistant but only to ampicillin. Reference site #2 yielded only one *E. coli* isolate from groundwater which was resistant to four antimicrobials – tetracycline, chlortetracycline, trimethoprim and ampicillin. This isolate came from a well near a surface water sampling site that was under reducing and low pH conditions.

Discussion

The information obtained from this study revealed that *E. coli* was found more frequently in groundwater on or near swine farms than on crop farms with no swine in eastern North Carolina. *E. coli* were found on a farm with a conventional anaerobic lagoon and sprayfield land application system, as well as on a farm with alternative technology consisting of separating, compacting and land applying the swine waste solids along with anaerobic lagoon liquid. These findings provided evidence of faecal contamination of the groundwater by swine farms. Swine farm #2 had higher concentrations of *E. coli* in the groundwater than swine farm #1. Swine Farm #2 was a smaller operation (design capacity for 1,500) with farrow to finish swine that separated/compacted swine waste solids and applied them to land without further treatment along with returning the separated liquid to the lagoon for subsequent storage and land application. The lack of lagoon storage of swine waste solids prior to land application at swine farm #2 may have resulted in higher loads of land applied *E. coli* that could penetrate into groundwater.

Swine farm #1 had sandy soils while swine farm #2 had well drained loamy sandy soils with moderate permeability. Differences in soils and in geo-hydrological conditions may also have contributed to differences in bacteria infiltration and transport through the soil matrix to groundwater on the different study sites. However, it was not possible to specifically investigate this. The detection of fewer *E. coli* bacteria at swine farm #1 site than at swine farm #2 could also be related to other factors that were not systematically investigated in this study (such as differences in timing/magnitude of land application of swine wastes and soil moisture content).

There were only very low levels of *E. coli* found in one well each during the first round of sampling at the reference sites. The one positive well at reference site #2 may have been due to (i) the combination of unique chemical constituents, (ii) vulnerability to surface water contamination and (iii) the presence of vegetation all of which may be factors contributing to *E. coli* presence and its possible persistence and growth. There were statistically significant differences among the *E. coli* frequencies and concentrations in groundwater between swine farm #2 and the other three sites and temporal variability was seen among the different sampling periods.

The results from this study showed that the frequency of occurrence of antimicrobial resistance traits in *E. coli* isolates from groundwater at swine farms was significantly higher than at the reference sites studied. Multi-drug resistance was present in *E. coli* isolates from groundwater near swine farm sites having lagoons and sprayfields with isolates resistant to 4–6 antimicrobials. This magnitude and frequency of multi-resistance was not seen in *E. coli* isolates at the reference sites. The antimicrobials to which resistance was observed in bacteria were generally consistent with the antimicrobials approved for use in swine feeding operations.

Conclusions

Overall, the results of this study demonstrated that antibiotic-resistant *E. coli* were present in groundwaters associated with commercial swine farms that have anaerobic lagoons and land application systems for swine waste management. Therefore, groundwater on or

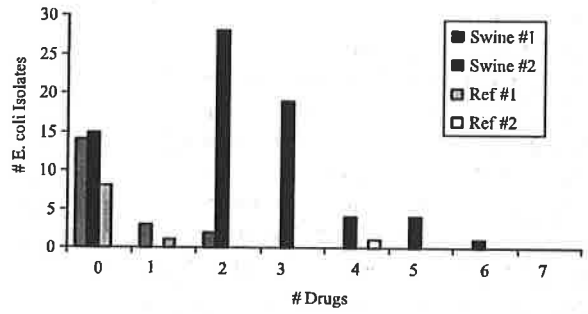


Figure 1 Frequency distribution of antibiotic resistance of groundwater *E. coli* isolates

$p = 0.003$). There were also significant differences between swine farm #2 and the reference sites (#1: $p = 0.03$; #2: $p = 0.009$). There was no significant difference in frequency of resistance traits/isolate between swine farm #1 site and the reference sites (#1: $p = 0.29$; #2: $p = 0.11$) and also with the two reference sites ($p = 0.37$). Overall, frequencies of antimicrobial resistance traits per *E. coli* isolate were significantly higher at swine farms than at reference sites.

MICs were generated for 17 antimicrobials of which it was expected that isolates would be inherently resistant to vancomycin, erythromycin, tiamulin, tylosin base, and clindamycin (Table 1). *E. coli* isolates from swine farm #1 had resistance to chlortetracycline, tetracycline and sulfamethoxazole (all approved for use in swine) (Figure 2). *E. coli* isolated from swine farm #2 site also had predominant resistance to tetracycline and chlortetracycline. The isolates were also resistant to ampicillin, streptomycin, chloramphenicol, sulfamethoxazole and trimethoprim, as well as florfenicol and neomycin. Of the antimicrobial resistance traits found in *E. coli* isolates, only chloramphenicol was an antibiotic not permitted for use in swine feed or to treat swine disease (it is a drug of last resort for human therapy). However, florfenicol is a derivative of chloramphenicol that is approved for use in cattle by the US FDA-CVM (Purdue Research Foundation, 1996). Cross-resistance between florfenicol and chloramphenicol has been seen in bovine *E. coli* isolates. The *E. coli* isolates in the study were resistant to florfenicol mediated by the flo gene, which specifies non-enzymatic cross-resistance to both florfenicol and chloramphenicol (White *et al.*, 2001). Trimethoprim can be used to treat sick animals (but is also used for humans) and sulfamethoxazole is primarily used in combination with

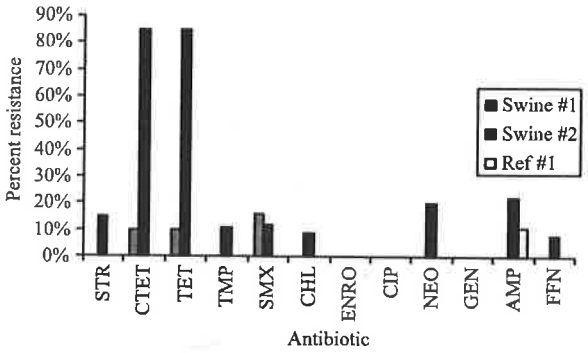


Figure 2 Drug resistance (%) of biochemically speciated groundwater *E. coli* isolates

results at the other sites. However, the shallower wells at 10 ft (3 m) at this site were representative of the surficial aquifer but were not positive for bacteria.

Only one *E. coli* isolate (0.5 CFU/100 mL) appeared in 1/6 wells at reference site #2 during the first round of sampling (September/October 2001) and not in the subsequent three rounds (April/May 2002, June 2002, August 2002). The one positive well was 8.9 ft (2.7 m) deep and was located only a few metres from the surface water on the site. A prior study at this site (Fanuel, 1999) revealed that the chemistry in this well was under reducing conditions, and this study confirmed low pH (4.5–5.2) and very low dissolved oxygen levels (<0.1 mg/L). The previous study also found that water samples from this well were chemically distinct from the other wells with significantly lower concentrations of calcium, magnesium, dissolved oxygen and more dissolved organic carbon, ammonium, and iron.

The Kruskall–Wallis χ^2 test was employed to determine if there were statistically significant differences in bacteria occurrence and concentrations in all of the groundwater samples among the four study sites. The analysis was applied to all groundwater samples for all farms including samples that were negative for *E. coli*. These analyses revealed that there were statistically significant differences in the *E. coli* concentrations between the two swine farms ($p = 0.003$). This was attributable to the higher *E. coli* concentrations present at swine farm #2 and may have been due to differences in numbers of animals, waste management practices and geo-hydrological or other environmental conditions. There were also statistically significant differences in *E. coli* concentrations between swine farm #2 and the reference sites (#1: $p = 0.003$; #2: $p = 0.005$). However, there were no statistically significant differences in *E. coli* concentrations when comparing the swine farm #1 to the references sites (#1: $p = 0.36$; #2: $p = 0.41$).

E. coli antimicrobial resistance

Knowing the antimicrobial usage at the two swine farms would facilitate efforts to determine if there was a direct positive association between antimicrobial use and the presence of antimicrobial resistant *E. coli* isolates in groundwater on or near these farms. However, information on antimicrobial usage was unavailable and even deemed proprietary when it comes to what antibiotics are included in the swine feed. What was known was that the following antimicrobials in the Sensititre panel used for antimicrobial testing were approved for use in swine feed – streptomycin, tetracycline, chlortetracycline, sulfamethoxazole, neomycin, tiamulin, and tylosin base and that the following antimicrobials were approved for use as a water soluble diet additive or for injection to treat disease – erythromycin, gentamicin and ampicillin.

Of the 19 *E. coli* isolates from swine farm #1, two were resistant to two antimicrobials, three were resistant to one antimicrobial and the rest were non-resistant. Hence, 7/19 isolates (37%) had antimicrobial resistance. Of the 71 *E. coli* isolates from swine farm #2, 56 (79%) had resistance with nine resistant to 4–6 antimicrobials, 47 resistant to 2–3 antimicrobials and 15 non-resistant. Only 1/10 *E. coli* isolates from reference site #1 (10%) and the only *E. coli* isolate from reference site #2 displayed antimicrobial resistance traits (Figure 1).

The Wilcoxon Exact Scores test was used to compare frequencies of occurrence of antimicrobial resistance traits in individual *E. coli* isolates. The null hypothesis was that there would be no differences in antimicrobial resistance trait frequencies in *E. coli* isolates based on their sites. There were not quite statistically significant differences (at the 5% confidence level or $p = <0.05$) in *E. coli* antimicrobial resistance trait frequencies between the two swine farms ($p = 0.06$). There were significant differences between the swine farms pooled together versus those at the reference sites (#1: $p = 0.01$; #2:

Results

E. coli occurrence in groundwater

A total of 134 presumptive *E. coli* were collected from the four sites with 100 confirmed as *E. coli* through biochemical testing. Swine farm #1 yielded 19 *E. coli* colonies in the groundwater. Only one *E. coli* colony was found in two wells each in the first round of sampling (March/May 2001): one well was located in the middle of a land application sprayfield and one well was located down gradient of the swine waste lagoon. The second round of sampling (February/April 2002) had higher *E. coli* concentrations with five positive wells and concentrations of 1–6 CFU/100 mL. The positive wells were two shallow wells located in the middle of sprayfields, two wells located at the edge of a sprayfield and one well located down gradient from the swine waste lagoon. The third round of sampling (May/June 2002) yielded only one *E. coli* colony that was located in one of the control wells up gradient of the swine farm. The fourth round (September 2002) yielded no *E. coli* colonies in the groundwater at all due to continuing drought conditions and several wells were unable to be sampled due to a lack of water in the wells. Overall, *E. coli* were found more often, and in higher concentrations, in areas associated with the land application of swine waste (sprayfields) and in wells located down gradient from the swine waste lagoon than in the control wells at swine farm #1.

Swine farm #2 gave 71 *E. coli* colonies in groundwater. The first round (May/June 2001) had one well and two piezometers positive for *E. coli* out of the 12 total. The well averaged 16.4 CFU/100 mL and one piezometer yielded only one *E. coli* colony (0.5 CFU/100 mL). The second piezometer had 1,045 CFU/100 mL and inspection of the piezometer proved that it might be a conduit for contamination in the aquifer. This piezometer was sampled again in the fourth round and yielded no *E. coli*. The second round (December 2001) had four wells and one piezometer positive for *E. coli* (0.5–32.7 CFU/100 mL). The piezometer was close to the sprayfield and stream, two positive wells were in the sprayfield and the other two wells were located down gradient from the swine waste lagoon. The third round (April 2002) yielded only one well positive for *E. coli* (1.8 CFU/100 mL) and the final round of sampling (September 2002) had three wells positive with *E. coli* at low levels (1.0–1.8 CFU/100 mL). Round one showed higher *E. coli* concentrations in groundwater than all other rounds. Round two also revealed positive *E. coli* colonies in the groundwater samples, but by rounds three and four the *E. coli* concentrations were at non-detectable levels in all wells due to summer and drought conditions similar to the findings at swine farm #1.

Low *E. coli* concentrations (4.5 CFU/100 mL) appeared in only 1/7 wells at reference site #1 during the first round of sampling (June/August 2001) and none were detected in the subsequent three sampling rounds (February 2002, May 2002, August 2002). The positive well was located off to the east of the rest of the wells and was not in line of the groundwater flow path that the rest of the wells represented. There was the potential for cattle manure to penetrate through the soil matrix from a small herd of beef cattle (approximately 10 animals) that were allowed to graze approximately 25 m up gradient from the location of the wells. Therefore, the results from this site may have indicated bacteria from cattle sources but the absence of detectable *E. coli* bacteria in groundwater samples from down gradient wells suggested that their waste did not have a major impact on groundwater microbial quality during the study period. This site was considered to be an agricultural reference site because it did not contain swine or their waste material. Most of the wells at reference site #1 were deeper (10–40 ft; 3–12 m) than at reference site #2 (7–18 ft; 2–6 m). The deeper wells on this site were located below an aquiclude and were not representative of the shallow groundwaters that were yielding positive

where corn, soybeans, wheat and tobacco were grown and there were a total of six groundwater wells.

Groundwater samples were collected aseptically as grab samples in sterile 4 L bottles and placed in coolers with freezer packs. The collection pump tubing was cleansed with 70% ethanol between samples to prevent cross-contamination of the water samples from well-to-well. Water samples were analysed for *E. coli* within 30 h of collection. Four seasonal rounds of groundwater samples at each site were collected and analysed for *E. coli* over the course of one and a half years. Water samples were filtered (47 mm, 0.45 μm cellulose ester filters in sterile membrane filter apparatus) with each membrane being placed on mFC agar and, after overnight incubation, transferred to nutrient agar with MUG (NA-MUG) and incubated according to standard procedures (APHA, 1998).

Selected *E. coli* colonies from NA-MUG were picked and streaked onto tryptic soy agar for purification and subsequent biochemical characteriation by Enterotube II (Becton Dickenson, Sparks, MD) using *E. coli* (ATCC 25922) as positive control for the confirmation tests. Confirmed *E. coli* isolates were subjected to antibiotic susceptibility testing using customised commercially prepared plates (Sensititre 18–24 Hour Susceptibility System, TREK Diagnostics Inc., Westlake, OH). Minimum inhibitory concentrations (MIC) were determined for 17 antimicrobials encompassing those commonly specified for human therapy and for veterinary use in swine and cattle (Table 1). *E. coli* isolates were first grown on tryptic soy agar and 3–5 colonies were picked and resuspended in 4 mL sterile deionised water to give a turbidity of 0.5 MacFarland units. This suspension (10 μL) was transferred to 50 mL Mueller Hinton broth. After vortexing, 50 μL were transferred to each well of the custom plate. The plate was then sealed and incubated at 37 °C for 18–24 h. The plates were read by scoring for a collection of bacteria at the bottom of a well as positive. Three positive control wells were included within the plate for quality control of the test; including *E. coli* (ATCC strain 25922).

Table 1 Antimicrobials, dilution ranges, and MIC breakpoints

Antimicrobial	Approved for use in			Abbreviation	Dilution range (μg/mL)	MIC breakpoints
	f	d	h			
Streptomycin	X		X	STR	32–1024	R ≥ 64; S ≥ 32
Vancomycin				VAN	8–256	R ≥ 32
Chlortetracycline	X			CTET	0.5–64	R ≥ 16; S ≥ 4
Tetracycline	X		X	TET	0.5–64	R ≥ 16; S ≥ 4
Trimethoprim			X	TMP	2–64	R ≥ 4; S ≥ 2
Sulfamethoxazole	X		X	SMX	32–1024	R ≥ 512; S ≥ 256
Chloramphenicol			X	CHL	4–128	R ≥ 32; S ≥ 8
Tiamulin	X			TIA	8–64	R ≥ 32
Erythromycin		X	X	ERY	4–32	R ≥ 8
Enrofloxacin				ENRO	1–16	R ≥ 4
Ciprofloxacin			X	CIP	1–16	R ≥ 4; S ≥ 1
Neomycin	X			NEO	16–128	R ≥ 32
Gentamicin		X		GEN	4–256	R ≥ 16; S ≥ 4
Ampicillin		X		AMP	8–128	R ≥ 32; S ≥ 8
Florfenicol				FFN	4–128	R ≥ 16
Tylosin Base	X			TYLB	5–80	R ≥ 16
Clindamycin			X	CLI	1–32	R ≥ 4

f = approved for use in swine feed (growth promotion); d = approved for use as injection or water soluble (disease treatment); h = approved for use in humans

recommended by the WHO (Edberg *et al.*, 1997; WHO, 2004). The half-life of *E. coli* is conservatively estimated to be at least 8 d under groundwater conditions. Therefore, the recovery of *E. coli* from a contamination event should be possible for weeks although the large dilution factor of aquifers must be taken into consideration (Edberg *et al.*, 1997).

Bacterial multi-drug and pan-resistance to antimicrobials is an important emerging public health issue. Resistant bacteria are spreading outside of hospitals, which are the traditional USA reservoir (CDC, 2002). Approximately half of the antibiotics produced globally flow into the agriculture industry and are used for infections and as growth promoters. High percentages of antimicrobial resistant bacteria in livestock waste and human exposure to agricultural animal faecal bacteria via food and occupational exposure have been documented (McDermott *et al.*, 2002). The range of pathways of exposure to these resistant bacteria must be established. Waterborne exposure is a possibility through drinking and recreational water that is contaminated with farm-origin antimicrobial resistant bacteria. The stability of resistant strains of bacteria in the environment has been demonstrated (Marshall *et al.*, 1990). Pigs were inoculated with an antimicrobial resistant strain of swine *E. coli* and the resistant strain was found in water, bedding materials, mice, flies and a human caretaker during the 4-month follow up period. Mathew *et al.* (1999) determined that resistance patterns differed between farm types and pigs of differing ages, indicating that pig age and degree of antibiotic use affect the resistance of faecal *E. coli*. Therefore, as antibiotic use changes, so do bacterial patterns of antibiotic resistance.

The purpose of this study was to quantify the presence of antibiotic-resistant *E. coli* in groundwater on commercial swine farms and to determine if there was a relationship between current swine waste management practices in eastern North Carolina and microbial impacts on groundwater quality.

Materials and methods

The four study sites, located in the Neuse River Basin in eastern North Carolina and having US Geological Survey (USGS) monitoring wells and known groundwater flow paths (Spruill, 2000), were screened for *E. coli* in groundwater. Swine farm #1 had swine from the feeder to finish stages and a design capacity for 5,000 animals. The swine waste was flushed from the barns with water and entered an anaerobic lagoon at the end of the barns, via pipes, where it was stored and partially treated prior to land application. The lagoon liquid was applied to fields surrounding the lagoons via pumps taking material directly from the upper level of the lagoon and applying it with a sprayer. There were 25 groundwater wells at this site including those located up and down gradient of the lagoon and in the middle of the sprayfields. Swine farm #2 was a farrow to finish swine operation with a design capacity for 1,500 animals. This facility had an alternative swine waste treatment technology in which the waste solids were removed from the barn flush and de-watered by compaction. The solids were applied to the field and the liquid stored in a lagoon prior to land application. There were 12 groundwater wells at this site, all located down-gradient of the swine barns and lagoon in relation to groundwater flow. The third site (reference #1) had crops such as corn, soybeans, and wheat growing on the field that formed the groundwater recharge area for the wells. Commercial fertiliser of unknown quantity was applied on two dates prior to the onset of sampling,and a small herd of beef cattle were allowed to graze approximately 25 m up gradient from the location of the wells. There were a total of seven groundwater wells at this site. The fourth site (reference #2) was a crop farm where there was no land application of swine waste or any agricultural animals present. The study site consisted of an upland field

Detection and occurrence of antimicrobially resistant *E. coli* in groundwater on or near swine farms in eastern North Carolina

M.E. Anderson and M.D. Sobsey

University of North Carolina at Chapel Hill, School of Public Health, Rosenau Hall CB#7400, Chapel Hill, NC 27599-7400, USA (E-mail: *meanders@oddpost.com*)

Abstract The use of antibiotics for growth promotion and disease treatment by the commercial swine industry has led to high proportions of multiple antibiotic-resistant enteric bacteria being shed by these animals and concerns about the environmental spread of these bacteria. A study was conducted to quantify the extent of release of antibiotic-resistant *E. coli* from swine farms into groundwater. Four study sites, two swine farms and two reference sites (crop farms), with known groundwater flow paths were screened for *E. coli* four times over the course of one and a half years. A total of 100 biochemically-confirmed *E. coli* were collected from the four sites. There were statistically significantly higher *E. coli* levels at the two swine farm sites than at the reference sites. The bacterial isolates were tested for antibiotic resistance using a panel of 17 drugs that are typical of human and veterinary use. There were 19 and 71 *E. coli* isolates from swine farms #1 and #2, respectively, with most (68%) being resistant to 1–6 antimicrobials. Only one *E. coli* isolate from each of the reference sites showed antimicrobial resistance traits. The results of this study demonstrate that antibiotic-resistant *E. coli* strains are present in groundwaters of swine farms with a typical lagoon and land application system for waste management.

Keywords Antibiotic resistance; *E. coli*; groundwater; lagoon; land application; swine

Introduction

The commercial swine industry and other sectors of animal agriculture use antibiotics for growth promotion and disease treatment, which has led to high proportions of multiply antibiotic-resistant enteric bacteria being shed by these animals. There are growing concerns about the spread of these bacteria through the food production chain and into the environment (Tollefson *et al.*, 1999; Teale, 2002). North Carolina is second in swine production in the USA with nearly 10 million animals raised on more than 3,000 farms (predominantly in the eastern coastal plain regions of the state). The porous soils and seasonally high water tables of this region can cause underlying groundwater to be vulnerable to surface contamination from a variety of sources including human and animal wastes. Previous studies have documented groundwater contamination by nitrates in the vicinity of swine farms, especially near the anaerobic lagoons in which swine waste is stored prior to periodic land application of the lagoon liquid (Stone *et al.*, 1998). Little is known about the extent to which swine farm wastes pose enteric microbial contamination risks to groundwater.

E. coli bacteria are universally present in faeces of all mammals at levels of over one billion/g faeces and the most faecal-specific member of the coliform group (Edberg *et al.*, 1997). Hence, *E. coli* is considered the definitive organism for demonstrating faecal pollution in water at least in temperate climates (Anon, 1987). Because *E. coli* provides evidence of a public health risk from enteric pathogens, there is zero tolerance for them in 100 mL volumes of drinking water. *E. coli* is considered a practical microbial indicator for public health protection and is

doi: 10.2166/wst.2006.471

Water Science & Technology Vol 54 No 3 pp 211–218

Food-borne origins of *Escherichia coli* causing extraintestinal infections. A.R. Manges, J.R. Johnson. *Clinical Infectious Diseases.* 2012. 55(5): 712-719.

Summary: A literature review of the strength of evidence linking human extraintestinal *Escherichia coli* (ExPEC) infections with a food-animal reservoir. Studies indicate a strong link between *E. coli* found in poultry and ExPEC strains recovered from humans, including genetic similarities and common antimicrobial-resistance patterns. Evidence reviewed demonstrates five of nine human ExPEC groups have also been identified in poultry, with one of these groups also found in pigs and cattle and one found also among pigs. Only three human ExPEC groups were determined to have no known food animal reservoir based on the available literature. Many of identified strains express extensive antibiotic-resistance, observed both among animals and humans. Authors indicate that although there are no known studies that can prove direct transmission between humans and food-animals, the weight of available evidence supports the presence of a food-animal reservoir for ExPEC. A discussion of public health interventions is also given.

Antimicrobial-resistant *Campylobacter* in the food chain in Mexico. M.B. Zaidi, P.F. McDermott, F. D. Campos, R. Chim, M. Leon, G. Vazquez, G. Figueroa, E. Lopez, J. Contreras, T. Estrada-Garcia. *Foodborne Pathogens and Disease*. 2012, 9(9): 841-847.

Summary: Describes the prevalence of *Campylobacter* from the intestines of food-animals at the time of slaughter, retail meat, and kindergarten-aged children in four regions of Mexico. Samples from chickens showed 94 percent of the 1,087 samples with *Campylobacter*. Seventy-one percent of 968 samples from swine, and 25 percent of 645 samples from cattle were also positive for *Campylobacter*. The same trend in retail meat was observed with 58, 15, and 5 percent of chicken, pork, and beef found to contain *Campylobacter*, respectively. Of 3,610 children with diarrhea 5 percent were found to be shedding *Campylobacter* as were 3 percent of asymptomatic children. Resistance to ciprofloxacin among *Campylobacter* from all sources was common with isolates from meat sources demonstrating the greatest proportion of resistance (approximately 85 percent) while a lower proportion of isolates from ill (62 percent) and healthy children (54 percent) were resistant to ciprofloxacin. Tetracycline resistance was also common (approximately 80 percent) among *Campylobacter* found in pork and beef and lower in ill (43 percent) and healthy children (37 percent). Resistance to other antimicrobials was also observed but at a lower rate. The presence of high proportions of resistance to ciprofloxacin and tetracycline observed among food-animals, meat, and children is of public health concern as fluoroquinolones are one of the drug classes of choice for treatment of severe *Campylobacter* infections in humans. Fluoroquinolones are not licensed for children and tetracyclines are prohibited for children under the age of eight years yet both of these antimicrobial classes are used in food-animal production in Mexico. This supports the role antimicrobial use has in food-animals contributing to antimicrobial resistance among human pathogens.

For additional information on the Pew Campaign on Human Health and Industrial Farming, or on any of these studies, please contact Laura Rogers, project director, Pew Health Group, at (202) 552-2018 or **lrogers@pewtrusts.org**.

Antibiotic resistance in foodborne pathogens: Evidence of the need for a risk management strategy. (CSPI White Paper). C. Smith DeWaal, C. Roberts, and C. Catella. Center for Science in the Public Interest, January 25, 2011.

Summary: Provides general background concerning the use of antibiotics in food animal-production and documents foodborne outbreaks due to antibiotic resistant bacteria. Focuses on documenting outbreaks due to antibiotic-resistant bacteria as antibiotic resistance is not required to be reported to other agencies in the U.S. Notes an increase in outbreaks due to antibiotic-resistant pathogens over the past several decades, although it is not clear whether this is due to a true increase or an increase in testing and reporting. A total of 35 outbreaks due to antibiotic-resistant pathogens were documented between 1973 and 2009. Source of the outbreaks were dairy products (34 percent), ground beef (26 percent), and poultry, pork, produce, and seafood (6 percent each), as well as eggs and multi-ingredient food (3 percent each). Outbreaks lead to 19,897 sick, 3.061 hospitalizations, and 26 deaths. *Salmonella typhimurium* was the most common pathogen implicated in outbreaks. Other *Salmonella* species were the causative agent in outbreaks as well as *Escherichia coli*, *Campylobacter jejuni,* and *Staphylococcus aureus*. These bacteria demonstrated a range of resistance patterns that included resistance to a total of 14 antibiotics including seven classified as "critically important" to human medicine by the World Health Organization.

Chicken as a reservoir for extraintestinal pathogenic *Escherichia coli* in humans, Canada. C.R. Bergeron, C. Prussing, P. Boerlin, D. Daignault, L. Dutil, R.J. Reid-Smith, G.G. Zhanel, A.R. Manges. *Emerging Infectious Diseases*. 2012. 18(3): 415-421.

Summary: Examined the potential for a food-animal reservoir for extraintestinal pathogenic *Escherichia coli* (ExPEC), a common cause of urinary tract infection (UTI) in humans. To address this question, researchers analyzed *E. coli* isolates obtained between 2005 and 2008 from humans with a diagnosed UTI, retail meat (chicken, beef, pork), and industrially raised food animals (chicken, beef cattle, pigs) in Canada. Fifteen distinct groups, containing 22 human isolates and 41isolates from retail meat were identified, with 71 percent of the retail meat isolates originating from chicken. Eight distinct groups, containing 17 human isolates and 29 isolates from animals at slaughter were identified, again with a majority of isolates from animals originating from chicken (79 percent). Three groups included isolates from all three sources. Among these distinct groups, genetic mapping of the isolates from humans, animals, and meat indicates that they may have originated from a recent common ancestor. The findings support the idea that food animals, and specifically chicken, may serve as a reservoir for ExPEC, allowing humans to become exposed through handling or consumption of retail chicken.

***Enterococcus faecalis* clones in poultry and in humans with urinary tract infections, Vietnam.** L.L. Poulsen, M. Bisgaard, N.T. Son, N.V. Trung, H.M. An, A. Dalsgaard. *Emerging Infectious Diseases*. 2012. 18(7): 1096-1100.

Summary: Presents information on *Enterococus faecalis* isolates from 31 humans with a urinary tract infection (UTI) and from poultry living in the same household as the infected individual. Sequence types (ST) of *E. faecalis* isolated from 23 percent (7/31) of UTI patients were identical to the types isolated from poultry living within the same household. For these seven pairs, the drug resistance patterns and the presence of virulence genes were also similar for human and poultry isolates. This report illustrates the potential for zoonotic transmission of *E. faecalis* between humans and poultry.

to a dairy farm, and the authors hypothesized that the infections were associated with handling or consuming the contaminated foods.

Many Studies Have Found That Transference of Antibiotic-Resistant Bacteria from Animals to Humans Is a Human Health Risk, but Researchers Disagree About the Extent of Risk

The extent of harm to human health from the transference of antibiotic-resistant bacteria from animals is uncertain. Many studies have found that the use of antibiotics in animals poses significant risks for human health, and some researchers contend that the potential risk of the transference is great for vulnerable populations. However, a small number of studies contend that the health risks of the transference are minimal.

Many Researchers Contend That Antibiotic Use in Animals Poses Significant Risk for Human Health

Some studies have sought to determine the human health impacts of the transference of antibiotic resistance from animals to humans. For example, the Food and Agriculture Organization of the United Nations (FAO), OIE, and WHO recently released a joint report based on the scientific assessment of antibiotic use in animals and agriculture and the current and potential public health consequences.[34] The report states that use of antibiotics in humans and animals alters the composition of microorganism populations in the intestinal tract, thereby placing individuals at increased risk for infections that would otherwise not have occurred. The report also states that use of antibiotics in humans and animals can also lead to increases in treatment failures and in the severity of infection.

Similarly, a recent review of studies regarding increased illnesses due to antibiotic-resistant bacteria found significant differences in treatment outcomes of patients with antibiotic-resistant bacterial infections and patients with antibiotic-susceptible bacterial infections.[35] For example, one study found that hospitalization rates of patients with nontyphoidal salmonella infections were 35 percent for antibiotic-resistant infections and 27 percent for antibiotic-susceptible infections. That study also found

[34]Food and Agriculture Organization of the United Nations, Office International des Epizooties, and World Health Organization, *Joint FAO/OIE/WHO Expert Workshop on Non-Human Antimicrobial Usage and Antimicrobial Resistance: Scientific Assessment* (Geneva, Switzerland, Dec. 1-5, 2003).

[35]Karin Travers and Michael Barza, "Morbidity of Infections Caused by Antimicrobial-Resistant Bacteria," *Clinical Infectious Diseases*, vol. 34, suppl. 3 (2002): S131-S134.

Researchers have also documented human infections caused by multidrug-resistant strains of salmonella linked to animals. In 1982, researchers used molecular subtyping to show that human isolates of multidrug-resistant salmonella bacteria were often identical or nearly identical to isolates from animals.[30] In the mid-1990s, NARMS data showed a rapid growth of multidrug resistance in *Salmonella enterica* serotype Typhimurium definitive type (DT) 104 among humans.[31] Molecular subtyping found that human isolates with this strain of multidrug resistance in *Salmonella enterica* serotype Typhimurium DT104 in 1995 were indistinguishable from human isolates with this strain tested in 1985 and 1990. These results indicated that the widespread emergence of multidrug resistance in *Salmonella enterica* serotype Typhimurium DT104 may have been due to dissemination of a strain already present in the United States. Because food animals are the reservoir for most domestically acquired salmonella infections and transmission from animals to humans occurs through the food supply, the researchers concluded that the human infections were likely from the animals.

Recently, there has been an emergence of multidrug-resistant *Salmonella enterica* serotype Newport infections that include resistance to cephalosporins,[32] such as cefoxitin.[33] Based on molecular subtyping, multidrug-resistant salmonella isolates from cattle on dairy farms were found to be indistinguishable from human isolates. An epidemiologic study found that the infections in humans were associated with direct exposure

[30]Thomas F. O'Brien, John D. Hopkins, Elaine S. Gilleece, Antone A. Medeiros, Ralph L. Kent, Billie O. Blackburn, Marion B. Holmes, Joseph P. Reardon, James M. Vergeront, Wendy L. Schell, Eleanor Christenson, Marjorie L. Bissett, and Erskine V. Morse, "Molecular Epidemiology of Antibiotic Resistance in Salmonella from Animals and Human Beings in the United States," *New England Journal of Medicine*, vol. 307, no. 1 (1982): 1-6.

[31]Efrain M. Ribot, Rachel K. Wierzba, Frederick J. Angulo, and Timothy J. Barrett, "*Salmonella enterica* serotype Typhimurium DT104 Isolated from Humans, United States, 1985, 1990, and 1995," *Emerging Infectious Diseases*, vol. 8, no. 4 (2002): 387-391.

[32]Cephalosporins are antibiotics that are commonly used, especially in children, to treat severe salmonella infections.

[33]Amita Gupta, John Fontana, Colleen Crowe, Barbara Bolstorff, Alison Stout, Susan Van Duyne, Mike P. Hoekstra, Jean M. Whichard, Timothy J. Barrett, Frederick J. Angulo, for the National Antimicrobial Resistance Monitoring System PulseNet Working Group, "Emergence of Multidrug-Resistant *Salmonella enterica* Serotype Newport Infections Resistant to Expanded-Spectrum Cephalosporins in the United States," *Journal of Infectious Diseases*, vol. 188 (2003): 1707-1716.

use of two new fluoroquinolones, one in humans in 1985 and one in animals in 1987, was responsible for the quinolone-resistant strains. The authors asserted that the extensive use of fluoroquinolones in poultry and the common route of campylobacter infection from chickens to humans suggest that the resistance was mainly due to the use of fluoroquinolones in poultry.

Salmonella Bacteria

Several epidemiologic studies using molecular subtyping have linked antibiotic-resistant salmonella infections in humans, another common foodborne illness, to animals. For example, in 1998 bacteria resistant to ceftriaxone were isolated from a 12-year-old boy who lived on a cattle farm in Nebraska.[27] Molecular subtyping revealed that an isolate from the boy was indistinguishable from one of the isolates from the cattle on the farm. No additional ceftriaxone-resistant salmonella infections were reported in that state or adjoining states that could have been the cause of the infection. Similarly, an epidemiologic study in Poland from 1995 to 1997 using molecular subtyping found identical profiles for ceftriaxone-resistant salmonella bacteria in isolates from poultry, feed, and humans.[28] The researchers concluded that the salmonella infections were introduced in the poultry through the feed and reached humans through consumption of the poultry. Researchers in Taiwan also found that *Salmonella enterica* serotype choleraesuis bacteria that were resistant to ciprofloxacin in isolates collected from humans and swine were closely related and, following epidemiologic studies, concluded that the bacteria were transferred from swine to humans.[29]

[27]Paul D. Fey, Thomas J. Safranek, Mark E. Rupp, Eileen F. Dunne, Efrain Ribot, Peter C. Iwen, Patricia A. Bradford, Frederick J. Angulo, and Steven H. Hinrichs, "Ceftriaxone-Resistant Salmonella Infection Acquired by a Child from Cattle," *New England Journal of Medicine*, vol. 342 (2000): 1242-1249.

[28]Andrzej Hoszowski and Dariusz Wasyl, "Typing of *Salmonella enterica* subsp. *enterica* serovar Mbandaka Isolates," *Veterinary Microbiology*, vol. 80 (2001): 139-148.

[29]Po-Ren Hsueh, Lee-Jene Teng, Sung-Pin Tseng, Chao-Fu Chang, Jen-Hsien Wan, Jing-Jou Yan, Chun-Ming Lee, Yin-Ching Chuang, Wen-Kuei Huang, Dine Yang, Jainn-Ming Shyr, Kwok-Woon Yu, Li-Shin Wang, Jang-Jih Lu, Wen-Chien Ko, Jiunn-Jong Wu, Feng-Yee Chang, Yi-Chueh Yang, Yeu-Jun Lau, Yung-Ching Liu, Cheng-Yi Liu, Shen-Wu Ho, and Kwen-Tay Luh, "Ciprofloxacin-Resistant *Salmonella enterica* Typhimurium and Choleraesuis from Pigs to Humans, Taiwan," *Emerging Infectious Diseases*, vol. 10, no. 1 (2004): 60-68.

the chromosome of campylobacter, the resistance is generally not transferred to other species of bacteria. Therefore when fluoroquinolone-resistant campylobacter bacteria are detected in human isolates, the source is likely to be other reservoirs of campylobacter bacteria, including animals. In some cases, molecular subtyping techniques have shown that fluoroquinolone-resistant isolates of campylobacter from food, humans, and animals are similar.

Fluoroquinolone-resistant *Campylobacter jejuni* in humans has increased in the United States and has been linked with fluoroquinolone use in animals. CDC reported that in the United States the percentage of *Campylobacter jejuni* in human isolates that were resistant to fluoroquinolones increased from 13 percent in 1997 to 19 percent in 2001.[24] A study in Minnesota found that fluoroquinolone-resistant *Campylobacter jejuni* was isolated from 14 percent of 91 chicken products obtained from retail markets in 1997.[25] Through molecular subtyping, the strains isolated from the chicken products were shown to be the same as those isolated from nearby residents, thereby bolstering the case that the chickens were the source of the antibiotic resistance.

During the 1980s, the resistance of campylobacter bacteria to fluoroquinolones increased in Europe. European investigators hypothesized that there was a causal relationship between the use of fluoroquinolones in animals and the increase in fluoroquinolone-resistant campylobacter infections in humans. For example, an epidemologic study that included molecular subtyping in the Netherlands found that among different strains of campylobacter bacteria, the percentage of fluoroquinolone-resistant strains in isolates tested had risen from 0 percent in both human and animal isolates in 1982 to 11 percent in human isolates and 14 percent in poultry isolates by 1989.[26] The authors concluded that the

[24]Centers for Disease Control and Prevention, *National Antimicrobial Resistance Monitoring System: Enteric Bacteria 2001 Annual Report* (2003): 10.

[25]Kirk E. Smith, John M. Besser, Craig W. Hedberg, Fe T. Leano, Jeffrey B. Bender, Julie H. Wicklund, Brian P. Johnson, Kristine A. Moore, Michael T. Osterholm, and the investigation team, "Quinolone-resistant *Campylobacter jejuni* Infections in Minnesota, 1992-1998," *New England Journal of Medicine*, vol. 340, no. 20 (1999).

[26]Hubert Ph. Endtz, Gijs J. Ruijs, Bert van Klingeren, Wim H. Jansen, Tanny van der Reyden, and R. Peter Mouton, "Quinolone Resistance in Campylobacter Isolated from Man and Poultry Following the Introduction of Fluoroquinolones in Veterinary Medicine," *Journal of Antimicrobial Chemotherapy*, vol. 27 (1991): 199-208.

There is also evidence to suggest that antibiotic-resistant enterococcus has developed from the use of antibiotics in animals. Vancomycin[21] resistance is common in intestinal enterococci of both exposed animals and nonhospitalized humans only in countries that use or have previously used avoparcin (an antibiotic similar to vancomycin)[22] as an antibiotic growth promoter in animal agriculture.[23] Since the EU banned the use of avoparcin as a growth promoter, several European countries have observed a significant decrease in the prevalence of vancomycin-resistant enterococci in meat and fecal samples of animals and humans.

Evidence Shows That Antibiotic-Resistant Campylobacter and Salmonella Bacteria Have Been Transferred to Humans

Epidemiologic studies that include molecular subtyping have demonstrated that antibiotic-resistant campylobacter and salmonella bacteria have been transferred from animals to humans through the consumption or handling of contaminated meat. That is, strains of antibiotic-resistant bacteria infecting humans were indistinguishable from those found in animals, and the authors of the studies concluded that the animals were the source of infection.

Campylobacter Bacteria

The strongest evidence for the transfer of antibiotic-resistant bacteria from animals to humans is found in the case of fluoroquinolone-resistant campylobacter bacteria. Campylobacter is one of the most commonly identified bacterial causes of diarrheal illness in humans. The strength of the evidence is derived in part from the fact that the particular way fluoroquinolone resistance develops for campylobacter bacteria makes it easier to identify the potential source of the resistance. Most chickens are colonized with campylobacter bacteria, which they harbor in their intestines, but which do not make them sick. Fluoroquinolones are given to flocks of chickens when some birds are found to have certain infections caused by *E. coli*. In addition to targeting the bacteria causing the infection, treatment of these infections with fluoroquinolones almost always replaces susceptible campylobacter bacteria with fluoroquinolone-resistant campylobacter bacteria. Because fluoroquinolone resistance is located on

[21]The antibiotic vancomycin has been reserved to treat infections, such as enterococcus infections, in humans that are resistant to antibiotics normally used for treatment.

[22]Avoparcin has never been approved for food animal use in the United States.

[23]Anthony E. van den Bogaard and Ellen E. Stobberingh, "Epidemiology of Resistance to Antibiotics Links between Animals and Humans," *International Journal of Antimicrobial Agents*, vol. 14 (2000): 327-335.

Epidemiologic Evidence Suggests That Patterns of Antibiotic Resistance in Humans Are Associated with Changes in Antibiotic Use in Animals

Evidence from epidemiologic studies that do not include molecular subtyping indicates that patterns of antibiotic resistance in humans are associated with changes in the use of particular antibiotics in animals. For example, work conducted in the United States in the 1970s showed an association between the use of antibiotic-supplemented animal feed in a farm environment and the development of antibiotic-resistant *E. coli* in the intestinal tracts of humans and animals.[18] In the study, isolates from chickens on the farm and from people who lived on or near the farm were tested and found to have low initial levels of tetracycline-resistant *E. coli* bacteria. The chickens were then fed tetracycline-supplemented feed, and within 2 weeks 90 percent of them were excreting essentially all tetracycline-resistant *E. coli* bacteria. Within 6 months, 7 of the 11 people who lived on or near the farm were excreting high numbers of resistant *E. coli* bacteria. Six months after the tetracycline-supplemented feed was removed, no detectable tetracycline-resistant organisms were found in 8 of the 10 people who lived on or near the farm when they were retested. Another study,[19] based on human isolates of *Campylobacter jejuni* submitted to the Minnesota Department of Health, reported that the percentage of *Campylobacter jejuni* in the isolates that were resistant to quinolone increased from approximately 0.8 percent in 1996 to approximately 3 percent in 1998.[20]

[18]Stuart B. Levy, George B. Fitzgerald, and Ann B. Macone, "Spread of Antibiotic-Resistant Plasmids from Chicken to Chicken and from Chicken to Man," *Nature*, vol. 260, no. 5546 (1976): 40-42; and Stuart B. Levy, George B. Fitgerald, and Ann B. Macone, "Changes in Intestinal Flora of Farm Personnel after Introduction of a Tetracycline-Supplemented Feed on a Farm," *New England Journal of Medicine*, vol. 295 (1976): 583-588.

[19]Kirk E. Smith, John M. Besser, Craig W. Hedberg, Fe T. Leano, Jeffrey B. Bender, Julie H. Wicklund, Brian P. Johnson, Kristine A. Moore, Michael T. Osterholm, and the investigation team, "Quinolone-resistant *Campylobacter jejuni* infections in Minnesota, 1992-1998," *New England Journal of Medicine*, vol. 340, no. 20 (1999).

[20]These percentages are from isolates from people who acquired the infections in the United States. There was a greater increase in the number of quinolone-resistant human isolates when infections acquired from foreign travel and from people who took fluoroquinolones prior to the collection of stool samples were included. Noting this, the percentage change between 1996 and 1998 of the domestically acquired infections was found to be statistically significant. FDA approved the use of fluoroquinolones in animals in 1995.

trade and protect local producers, unless that regulation is scientifically documented.

Antibiotic-Resistant Bacteria Have Been Transferred from Animals to Humans, but Researchers Disagree About the Extent of Potential Harm to Human Health

Research has shown that antibiotic-resistant bacteria have been transferred from animals to humans, but the extent of potential harm to human health is uncertain. Evidence from epidemiologic studies suggests associations between patterns of antibiotic resistance in humans and changes in antibiotic use in animals. Further, evidence from epidemiologic studies that include molecular subtyping to identify specific pathogens has established that antibiotic-resistant campylobacter and salmonella bacteria are transferred from animals to humans. Many of the studies we reviewed found that this transference poses significant risks for human health. Researchers disagree, however, about the extent of potential harm to human health from the transference of antibiotic-resistant bacteria.

Antibiotic-Resistant Bacteria Have Been Transferred from Animals to Humans

Antibiotic-resistant bacteria have been transferred from animals to humans. Evidence that suggests that this transference has taken place is found in epidemiologic studies showing that antibiotic-resistant *E. coli* and campylobacter bacteria in humans increase as use of the antibiotics increases in animals. Evidence that establishes transference of antibiotic-resistant bacteria is found in epidemiologic studies that include molecular subtyping. These studies have demonstrated that antibiotic-resistant campylobacter and salmonella bacteria have been transferred from animals to humans through the consumption or handling of contaminated meat. That is, strains of antibiotic-resistant bacteria infecting humans were indistinguishable from those found in animals, and the researchers concluded that the animals were the source of infection.

Maharjan, S. Mushtaq, T. Noorie, D.L. Paterson, A. Pearson, C. Perry, R. Pike, B. Rao, U. Ray, J.B. Sarma, M. Sharma, E. Sheridan, M.A. Thirunarayan, J. Turton, S. Upadhyay, M. Warner, W. Welfare, D.M. Livermore, and N. Woodford. *The Lancet*, 2010. 10(9): 597-602.

Summary: Presents information about antibiotic resistance in gram-negative bacteria. Fewer antibiotic agents exist to treat gram-negative bacterial infections and therefore resistance to antibiotics among these bacteria may be especially concerning. Antibiotic-resistance is shared by bacteria mainly through the transfer of plasmids (a mobile piece of DNA). After the discovery of CTX-M-15 extended-spectrum β-lactamase (ESBL), which confers resistance to cephalosporins, was reported in India, a greater reliance on carbapenems to treat infection has been observed. The article presents molecular and epidemiologic information on New Delhi metallo-β-lactamase 1 (NDM-1) positive Enterobacteriaceae in India, Pakistan, and the United Kingdom in 2008 and 2009. NDM-1 is a recently identified carbapenem resistance gene which has been shown to readily transfer between bacteria in vitro. Of 3,521 Enterobacteriaceae recovered in Chennai in 2009, 141 were carbapenem resistant, and 44 were positive for NDM-1 (1 percent of all isolates). In Haryana, 47 of 198 isolates were carbapenem resistant and 26 of those were positive for NDM-1. All 44 isolates from Chennai were resistant to all β-lactam antibiotics, fluoroquinolones, and aminoglycosides (except two susceptible to gentamicin) and came from community acquired urinary tract infections, pneumonia, and blood-stream infections. Isolates from the UK were mostly from samples from urine, blood, burn or wound, sputum, central line tip, and throat swabs. All UK NDM-1 positive isolates were resistant to imipenem and ertapenem. A majority of NDM-1 positive isolates from the UK and Chennai carried the NDM-1 gene on plasmids that could be transferred between bacteria. Isolates from Haryana were not transferable. The authors conclude Enterobacteriaceae with the NDM-1 enzyme are resistant to many antibiotic classes and may present a great deal of difficulty in treating Gram-negative infections with available drugs.

Association between antimicrobial resistance in *Escherichia coli* isolates from food animals and blood stream isolates from humans in Europe: An ecological study. A.R. Vieria, P. Collignon, F.M. Aarestrup, S.A. McEwen, R.S. Hendriksen, T. Hald, and H.C. Wegener. *Foodborne Pathogens and Disease*. 2011. Sep 1. [Epub ahead of print].

Summary: Estimates the correlation between antimicrobial resistance of *Escherichia coli* from human blood stream infections and from *E. coli* isolated from poultry, pigs, and cattle in eleven European countries. Reports strong correlations between antimicrobial resistance found in *E.coli* from human blood stream infections and poultry and human blood stream infections and pigs. States that in addition to contributions from human use of antimicrobials, a proportion of resistant *E. coli* implicated in human blood infections may be from food animal sources.

Selection of resistant bacteria at very low antibiotic concentrations. E. Gullberg, S. Cao, O.G. Berg, C. Ilbäck, L. Sandegren, D. Hughes, and D.I. Anderson. *PLoS Pathogens*. 2011. 7(7). E1002158.

Summary: Demonstrates through experiments with *Eschericia coli* and *Salmonella enterica* that the presence of levels of tetracycline and streptomycin several-hundred fold lower than previously considered important, may lead to selection for resistant strains of these bacteria over susceptible strains. The authors state that very low levels of antibiotics, which can be found in the environment or in the human body when undergoing treatment, are important in the development and maintenance of antibiotic resistance in pathogens.

in close contact with contaminated food, there is a risk of picking up antibiotic resistant *E. coli* that could lead to UTIs that are more difficult to treat.

Food reservoir for *Escherichia coli* causing urinary tract infections. C. Vincent, P. Boerlin, D. Daignault, C.M. Dozois, L. Dutil, C. Galanakis, R.J. Reid-Smith, P-P. Tellier, P.A. Tellis, K. Ziebell, and A.R. Manges. Emerging Infectious Diseases, 2010. 16(1):88-95.

Summary: The design of this study was to see if a food reservoir exists for *E. coli* that may cause urinary tract infections. Sampling for *E. coli* was completed between 2005 and 2007 comprising clinical UTI samples, retail meats and restaurant/ready-to-eat foods. Upon comparison of these collected isolates by molecular methods the author's report that *E. coli* identified from retail chicken and other food sources are identical or nearly the same as those from human UTIs.

***Escherichia coli* isolates from broiler chicken meat, broiler chickens, pork, and pigs share phylogroups and antimicrobial resistance with community-dwelling humans and patients with urinary tract infection.** L. Jakobsen, A. Kurbasic, L. Skjøt-Rasmussen, K. Ejrnaes, L.J. Porsbo, K. Pedersen, L.B. Jensen, H.D. Emborg, Y. Agerso, K.E.P. Olsen, F.M. Aarestrup, N.Frimodt-Moller, and A.M. Hammerum. *Foodborne Pathogens and Disease,* 2010. 7:5 537-547

Summary: Study in Denmark comparing phylogroups and antimircrobial resistance patterns among *E. coli* collected from UTI patients, community-dwelling humans, broiler chicken meat, broiler chickens, pork meat and pigs. The study finds that the presence of specific *E. coli* phylogroups, that are the main cause of UTIs, exist in samples of animal origin. The collected animal isolates also have similar antibiotic-resistance patterns as those collected from UTI patients and community-dwelling humans suggesting that food animals and meat may be a source of such isolates to humans. Samples from humans were predominantly B2, which is the most commonly found type in UTIs, most likely due to virulence factors associated with the group allowing colonization in humans. Only 6 to 15 percent of isolates of animal origin were found to fall into group B2, but these may still pose a risk for acquiring uropathogenic *E. coli.*

Risk factors for antibiotic-resistant *Escherichia coli* carriage in young children in Peru: Community –based cross sectional prevalence study. H.D. Kalter, R.H. Gilman, L.H. Moulton, A.R. Cullotta, L. Cabrera, and B. Velapatiño . *American Journal of Tropical Medicine and Hygiene,* 2010.

Summary: A study in Peru focused on the carriage and antimicrobial resistance characteristics of *E. coli* from children and their living environments that included animals, market chickens and mothers' hands. The study concludes that data from surveys and sampling for *E. coli* in several regions of Peru shows there were four main factors contributing to antibiotic-resistant *E. coli* carriage in children. Use of antibiotics by anyone in the household increased risk. Residing in an area where a larger proportion of households served home-raised chicken seemed to protect against resistant bacteria, however residing in an area that severed market-raised chicken was a risk factor for carriage of resistant *E. coli.* Also, living in environments contaminated with a higher level of multi-drug resistant bacteria were found to increase the risk of carriage of resistant *E. coli.*

Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: A molecular, biological, and epidemiological study. K.K. Kumarasamy, M.A. Toleman, T.R. Walsh, J. Bagaria, F. Butt, R. Balakrishnan, U. Chaudhary, M. Doumith, C.G., Giske, S. Irfan, P. Krishnan, A.V. Kumar, S.

Summary: Assesses the attributable cost associated with antimicrobial-resistant infections (ARI). Data were collected from patients admitted to a public teaching hospital in the Chicago area in the year 2000. Of 188 patients that met eligibility of ARI, the attributable medical cost of treatment ranged from $18,588 to $29,069 per patient. Social costs were $10.7 to $15.0 million, and total cost corrected to 2008 dollars was $13.35 million.

World health organization ranking of antimicrobials according to their importance in human medicine: A critical step for developing risk management strategies for the use of antimicrobials in food production animals. P. Collignon, J.H. Powers, T.M. Chiller, A. Aidara-Kane, and F.M.Aarestrup. *Clinical Infectious Diseases*. 2009. 49: 132-41.

Summary: Presents information regarding antimicrobial agents used to treat disease in humans. Ranks antimicrobial agents and classes as critically important, highly important, and important to human health and reviews changes in the rankings made in 2007. Antimicrobial rankings are based on two main criteria: 1) the agent or class is the sole therapy or one of few alternatives to treat serious human disease; 2) the antimicrobial agent or class is used to treat diseases cause by organisms that may be transmitted via nonhuman sources or diseases caused by organisms that may acquire resistance genes from nonhuman sources. Within the list of critically important antimicrobial agents a committee designated quinolones, third- and fourth-generation cephalosporins and macrolides as the classes for which immediate action should be taken to reduce unnecessary use in food animals and humans.

Antibiotic management of Staphylococcus aureus infections in US children's hospitals, 1999-2008. J.C. Herigon, A.L Hersh, J.S. Gerber, T.E. Zaoutis, and J.G. Newland. *Pediatrics*, 2010. 125:e1294-e1300.

Summary: This study focuses on the rates of *S. aureus* infection in children under the age of 18 from 1999 until 2008. The authors also track the trend of antimicrobial use during that time period. Finds that *S. aureus* infections increased by a rate of more than 10-fold over the course of 10 years from 14.8 per 1000 admissions in 1999 to 35.7 per 1000 admissions in 2008. MRSA infections also increased 10-fold during the same period from 2.0 cases per 1000 admissions in 1999 to 20.7 cases per 1000 admissions in 2008. Increased use of clindamycin was most substantial (21 percent in 1999 to 63 percent in 2008) while linezolid also saw increased use between 2001 (when it became available) and 2008. The substantial use of clindamycin may lead to greater resistance and ineffective treatment of future *S. aureus* infections. The authors note that continuous monitoring of local *S. aureus* susceptibility patterns is needed as treatment patterns have changed over the past decade due to the emergence of community-associated MRSA.

Genetic identity of aminoglycoside-resistance genes in *Escherichia coli* isolates from human and animal sources. P. Ho, R.C. Wong, S.W. Lo, K. Chow, S.S. Wong, and T. Que. *Journal of Medical Microbiology*, 2010. 59: 702-707.

Summary: A study in Hong Kong on *E. coli* isolates collected from food producing animals and humans (most from urinary tract infections). The group looked at the aminoglycocide (gentamicin) resistance characteristics of these isolates and found the main source of resistance was due to a gene called aacC2. The aacC2 gene was shown to exist in both human and animal *E. coli*. This suggests that gentamicin resistance in human *E. coli* urinary isolates can be attributed to resistance genes that are present in food-producing animals. Study illustrates when humans are

cow grouped with other human isolates collected from urinary tract infections and bacteremia . This shows that *E. coli* from animals may be a cause of UTIs and bactermia in humans.

Low-level fluoroquinolone resistance among *Camplyobacter jejuni* isolates in Australia. L. Unicomb, J. Ferguson, R.J. Stafford, R. Ashbolt, M.D. Kirk, N.G. Becker, M.S. Patel, G.G. Gilbert, M. Valcanis, and L. Mickan. *Clinical Infectious Diseases,* 2006. 42: 1368-1374.

Summary: Reports a study from five Australian states between 2001 and 2002 that looked into the susceptibility patterns of *Campylobacter jejuni*. Only two percent of isolates from locally acquired infections were resistant to ciprofloxacin, likely reflecting Australia's policy of restricting the use of fluoroquinolones in food production animals.

First report of the emergence of CTX-M-type extended spectrum ß-Lactamases (ESBLs) as the predominant ESBL isolated in a U.S. health care system. J. S. Lewis II, M. Herrena, B. Wickes, J.E. Patterson, and J. H. Jorgensen. Antimicrobial Agents and Chemotherapy, 2007. 51(11): 4015-4021.

Summary: A study on Extended spectrum beta-lactamases (ESBLs) from a clinic in San Antonio Texas. ESBLs are enzymes produced by bacteria that can negate the use of certain newer antibiotics used in treating infections of *E. coli* or similar bacteria. The new ESBL enzyme described here as seen for the first time in the U.S. is located on a plasmid (a mobile element of DNA) within the bacterium. As plasmids can be readily passed between bacteria this new finding could have a wide health impact. The authors state "a worrisome trend with the emergence of these enzymes has been an increasing frequency of *E. coli* isolates from outpatients or patients hospitalized for a very brief period, suggesting community acquisition of these strains."

Endemic and epidemic lineages of *Escherichia coli* that cause urinary tract infections. A.R. Manges, H. Tabor, P. Tellis, C. Vincent, and P. Tellier. *Emerging Infectious Diseases,* 2008. 14(10): 1575-1583.

Summary: Studies urinary tract infections (UTI) in women from California and Canada. Relatedness of the infections is apparent, as the profiles of the bacteria are identical. Multidrug-resistant *E. coli* outbreaks are the causative agent of the disease, and how these bacteria are acquired by the gut is unclear; however, the authors cite a previous study indicating that poultry and pork consumption may lead to the development of drug-resistant UTIs.

Temporal changes in the prevalence of community-acquired antimicrobial-resistant urinary tract infection affected by *Escherichia coli* clonal group composition. S.P. Smith, A.R. Manges, and L.W. Riley. Clinical Infectious Diseases, 2008. 46: 689-695.

Summary: Reports on urinary tract infections (UTIs) from 1,667 patients over the course of 6 years. *E. coli* specimens were collected and characterized by molecular methods. Twelve percent of human UTI samples collected were found to be from a specific group, which from previous work has been shown to include *E. coli* that had been collected from food animals or retail poultry products. The collected human isolates were also shown to be resistant to trimethoprim-sulfamethoxazole at a rate of 49 percent. The authors suggest that contaminated food products may be a source of drug resistant UTIs.

Hospital and societal costs of antimicrobial-resistant infections in a Chicago teaching hospital: Implications for antibiotic stewardship. R.R. Roberts, B. Hota, I. Ahmad, R.D. Scott II, S.D. Foster, F. Abbasi, S. Schabowski, L.M. Kampe, G.G. Ciavarella, M. Supino, J. Naples, R. Cordell, S.B. Levy, and R.A. Weinstein. *Clinical Infectious Diseases,* 2009. 49: 1175-1184.

mechanisms. Moderate amounts of cross-resistance to all three antibiotics were also detected. Concludes that exposure to low levels of antibiotics poses a risk and resistance selected for in the agricultural sector will transfer to the human sector over time and this transfer is already occurring.

Fluoroquinolone resistance in *Camplyobacter* absent from isolates, Australia. L. Unicomb, J. Ferguson, T.V. Riley, and P. Collignon. *Emerging Infectious Diseases,* 2003. 9(11): 1482-1483.

Summary: Reports on a study of fluoroquinolone resistance in New South Wales, Australia, over a three-year period. Only 12 *Campylobacter* isolates were found to be resistant to fluoroquinolones. Ten of these were related to travel; travel status of the other two is unknown. Australia has never allowed the use of fluoroquinolones in food animal production, a policy that may have impacts on human health for countries with fluoroquinolone-resistant cases of *Campylobacter.*

Antibiotic selection pressure and resistance in Streptococcus pneumoniae and Streptococcus pyogenes. W.C. Albrich, D.L. Monnet, and S. Harbarth. *Emerging Infectious Diseases,* 2004. 10:3 514-517.

Summary: Study designed to assess emerging antibiotic resistance in *Streptococcus pneumoniae* and *Streptococcus pyogenes* in 20 countries by comparing resistance rates to the dose of antibiotics given to outpatients. The authors find that resistance to penicillin and macrolides in these species in outpatients is directly correlated with increased antibiotic selection pressure on a national level and suggest that these findings lend support to policymakers and professional organizations to discourage the overuse of antibiotics in the community.

Possible animal origin of human-associated, multidrug-resistant, uropathogenic *Escherichia coli.* M. Ramchandi, A.R. Manges, C. DebRoy, S.P. Smith, J.R. Johnson, and L.W. Riley. *Clinical Infectious Disease,* 2005. 40: 251-257.

Summary: Reviews a collection of 495 animal and environmental *E. coli* isolates collected by the Gastroenteric Disease Center and determines that 26 percent had indistinguishable characteristics from human isolates. Concludes that the data suggest that drug-resistant, uropathogenic, human-associated *E. coli* strains may have an animal origin and that drug-resistant urinary tract infections in humans could be derived from foodborne illnesses.

The rising influx of multidrug-resistant gram-negative bacilli into a tertiary care hospital. A.E. Pop-Vicas, E. M. and C. D'Agata. *Clinical Infectious Diseases*, 2005. 40: 1792-8.

Summary: Studies multi-drug resistant (MDR) *E. coli*, *Klebsiella* species, *Enterobacter cloacae*, and *Pseudomonas aeruginosa* isolates from patients harboring these bacteria upon entering a hospital in Israel (within 48 hours of admittance). Finds that between 1998 and 2003 the prevalence of MDR isolates of all listed species increased significantly except *Pseudomonas aeruginosa.* Of the 464 isolates collected 12 percent, 35 percent and 53 percent were resistant to 5, 4 and 3 antimicrobial groups, respectively.

Analysis of a uropathogenic *Escherichia coli* clonal group by multilocus sequence typing. S.Y. Tartof, O.D. Solberg, A.R. Manges, and L.W. Riley. *Journal of Clinical Microbiology*, 2005. 5860-5864.

Summary: Forty-five strains of uropathogenic *E. coli* were analyzed by a molecular typing method called multi-locus sequence typing (MLST). The research shows that one sample from a

ANTIMICROBIAL-RESISTANT INFECTIONS

Infections arising with implications toward the use of antimicrobials in food animal production.

Molecular epidemiology of antibiotic resistance in *Salmonella* from animals and human beings in the United States. T.F. O'Brien, J.D. Hopkins, E.S. Gilleece, A.A. Medeiros, R.L. Kent, B.O. Blackburn, M.B. Holmes, J.P. Reardon, J.M. Vergeront, W.L. Schell, E. Christenson, M.L. Bisset, and E.V. Morse. *New England Journal of Medicine* 1982. 307:8 1-6.

Summary: Restriction-endonuclease digestion (a method by which DNA is cleaved at specific locations, then these digestion patterns are observed by gel-electrophoresis to compare similarity between samples) was used to analyze plasmids from *Salmonella* isolates collected from animals and humans. Results show that identical or nearly identical antibiotic resistance gene carrying plasmids are found between human and animal strains of *Salmonella*. Plasmid fragments were found not to cluster by human or animal grouping, rather they are intermixed suggesting that the strains developed in one host then were spread to the other, as both share similar characteristics. The infected patients observed had no prior farm exposure, this leaves meat or food preparation as a plausible route for infection, and also points toward the spread of disease from animals to humans.

Widespread distribution of urinary tract infections caused by a multidrug-resistant *Escherichia coli* clonal group. A.R. Manges, J.R. Johnson, B. Foxman, T.T. O'Bryan, K.E. Fullerton, and L.W. Riley. *New England Journal of Medicine,* 2001. 345(14): 1007-1013.

Summary: Studies urinary tract infections (UTIs) in the U.S. caused by *E. coli* resistant to trimethoprim–sulfamethoxazole as well as other antibiotics. Concludes that UTIs may be caused by contaminated foods, as the outbreaks appear to follow a pattern similar to that of *E. coli* O157 as they spread throughout a community.

De Novo* acquisition of resistance to three antibiotics by *Escherichia coli. M.A. van der Horst, J.M. Schuurmans, M.C. Smid, B.B. Koenders, and B.H. ter Kuile. *Microbial Drug Resistance.* 2001, 17(2): 141-147.

Summary: Explores *de novo* acquisition of resistance by *Escherichia coli* bacteria after varying levels of exposure to three antibiotics – amoxicillin, enrofloxacin, and tetracycline. *E. coli* samples were exposed to sub-lethal concentrations of each antibiotic. If normal growth occurred, colonies were selected and re-plated and exposed to a concentration of the antibiotic at a level doubling the previous exposure. *E. coli* grown in the absence of the amoxicillin had a minimum inhibitory concentration (MIC) that varied between 4 and 8 μg/ml. When grown with 1.25 or 2.5 μg/ml, the MIC reached a maximum of 32 μg/ml, and when grown for another 15 days without antibiotics, the MIC returned to control levels. However, colonies exposed to increasing amounts of amoxicillin reached a maximum MIC of 512 μg/ml and maintained a MIC of 256 μg/ml when grown without antibiotics for another 15 days demonstrating the ability to maintain resistance to amoxicillin. *E. coli* grown in the presence of tetracycline had increasing MIC levels with those exposed to increasing levels of tetracycline demonstrating a maximum MIC of 32 μg/ml. However, all MICs returned to control level when tetracycline was removed. Resistance among *E. coli* exposed to levels of enrofloxacin below the susceptible MIC built quickly, increasing by a factor of up to 100. The increased MIC remained after 15 days of growth in the absence of enrofloxacin. Growth rate of *E. coli* was also examined for each scenario. Differences in resistance and growth rate between the three antibiotics may be due to differences in resistance

similar. *S. aureus* isolated from cow farmers and non-farmers were similar to each other but different than those from pigs and pig farmers. A greater proportion of *S. aureus* isolates from pig farmers and veterinarians were resistant to antibiotics, especially tetracycline and similar to the resistance patterns in pigs. These results support the idea that zoonotic transmission of antimicrobial-resistant *S. aureus* may occur frequently between pigs and caretakers.

A metapopulation model to assess the capacity of spread of methicillin-resistant *Staphylococcus aureus* ST398 in humans. T. Porphyre, E.S. Giotis, D. H. Lloyd, K. Dorothea, C. Stärk. *PLoS One.* 2012. 7(10).

Summary: A mathematical model was used to investigate the ability of methicillin-resistant *Staphylococcus aureus* (MRSA) ST 398, a livestock-associated sequence type, to spread into a hypothetical human population from a commercial pig farm. Results showed that repeated exposures of humans working in direct contact with pigs carrying MRSA ST398, allowed for MRSA ST398 to persist in the human population even at low levels of persistence. Based on the results, the authors recommend farm-level interventions to reduce exposure to MRSA ST398 in order to control spread of MRSA ST398 in the greater population.

Valentine Leukocidin virulence gene. All MRSA isolates were resistant to penicillin, oxacillin, and tetracycline.

Methicillin resistant Staphylococcus aurues ST398 in veal calf farming: Human MRSA carriage related with animal antimicrobial usage and farm hygiene. H. Graveland, J.A. Wagenaar, H. Heesterbeek, D. Mevius, E. van Duijkeren, and D. Heederik. *PLoS One,* 2010. 5(6): 1-6.

Summary: Studies MRSA ST398 carriage in veal calves, farmers, their family members and employees. A large sampling size of veal calf farms in the Netherlands was selected at random to be screened for ST398. All participants were given a questionnaire to fill in describing their contact and role on the farm as well as how farm operations were conducted. Samples from both humans and veal calves were cultured and categorized using molecular techniques. The data presented show that direct associations between human and animal carriage of MRSA ST398 exist and that carriage was shown to increase in calves as antibiotic use on the farm increased. Duration of contact to veal calves showed a highly elevated risk of MRSA ST398 carriage in humans and a decrease in MRSA was seen in farms with better hygiene practices (ie cleaning of stables before new claves were brought on the farm). Disinfection was applied in less than 20 percent of the farms in the study and was not associated with prevalence of MRSA carriage in calves. Overall the prevalence of MRSA was 15.9 percent in participants who lived or worked on veal calf farms, which is far greater than the general population carriage rate in the Netherlands estimated to be below 1 percent.

***Staphylococcus aureus* CC398: Host adaptation and emergence of methicillin resistance in livestock.** L.B. Price, M. Stegger, H. Hasman, M. Aziz, J. Larsen, P.S. Andersen, T. Pearson, A.E. Waters, J.T. Foster, J. Schupp, J. Gillece, E. Driebe, C.M. Liu, B. Springer, I. Zdovc, A. Battisti, A. Franco, J. Zmudzki, S. Schwarz, P. Butaye, E. Jouy, C. Pomba, M. Concepcion Porrero, R. Ruimy, T.C. Smith, D.A. Robinson, J.S. Weese, C.S. Arriola, F. Yu, F. Laurent, P. Keim, R. Skov, F.M. Aarestrup. *mBio*. 2012. 3(1).

Summary: Authors applied whole genome sequence typing (WGST) to 89 CC398 *Staphyloccocus aureus* isolates from around the world and examined the origins and evolution of CC398. The article provides phylogenetic evidence that suggests that CC398 originated in humans as a methicillin susceptible *S. aureus* (MSSA) strain. Authors further suggest that this strain spread to livestock where it then acquired the SCC*mec* cassette and resistance to methicillin and tetracycline. Results presented also suggest that the transfer between humans and animals was followed by a reduced capacity for human colonization, transmission, and virulence despite the fact that MRSA CC398 is thought to be associated with an increase in MRSA infections in parts of Europe.

Antimicrobial resistance of *Staphylococcus aureus* strains acquired by pig farmers from pigs. A. Oppliger, P. Moreillon, N. Charrière, M. Giddey, D. Morisset, O. Sakwinska. *Applied Environmental Microbiology*. 2012. 78(22): Epub ahead of print.

Summary: Examines the genotype and antimicrobial resistance patterns of *Staphylococcus aureus* isolated from pigs, pig farmers, and veterinarians on 41 farms in Western Switzerland. This information is compared to characteristics of *S. aureus* isolated from people with no agricultural exposures and from cow farmers. Of 343 pigs tested, 123 (36 percent) were found to carry *S. aureus* while 44/75 pig farmers and veterinarians carried *S. aureus*. Eleven pigs (3 percent) from three farms were positive for MRSA as were five farmers (7 percent). The *S. aureus* found among pigs and pig farmers and veterinarians was

Summary: MRSA strains were found in 23 percent of the farms tested. States that the use of standard antimicrobials "seems to be a risk factor for finding MRSA-positive pigs on a farm. Pig farms on which the pigs were treated with antimicrobials as group medication had a higher risk of being MRSA positive, whereas farms on which antimicrobials were used restrictively had a much lower chance of being MRSA positive."

Increase in a Dutch hospital of methicillin-resistant *Staphylococcus aureus* related to animal farming. M.M.L. van Rijen, P.H. Van Keulen, and J.A. Kluytmans. *Clinical Infectious Diseases*, 2008. 16:261-263.

Summary: Reports on a study 2002–2006 in the Netherlands involving hospital patients who had MRSA. Patients exposed to pigs or veal calves were shown to be at higher risk for MRSA as there was an emergence of nontypable MRSA during this time. Nontypable MRSA is assumed to stem from pigs and calves.

Methicillin-resistant *Staphylococcus aureus* (MRSA) strain ST398 is present in Midwestern U.S. swine and swine workers. T.C. Smith, M.J. Male, A.L. Harper, J.S. Kroeger, G.P. Tinkler, E.D. Moritz, A.W. Capuano, L.A. Herwaldt, and D.J. Diekema. *PLoS ONE,* 2009. 4(1): e4258.

Summary: Investigates MRSA in the Midwestern U.S. Samples were taken from swine and production workers in two commercial operations. MRSA prevalence was 49 percent in swine and 45 percent in workers. Results show that MRSA is common in swine production in the U.S. and that these animals could be harboring the bacterium.

Methicillin-resistant *Staphylococcus aureus*: A new zoonotic agent? B. Springer, U. Orendi, P. Much, G. Hoger, W. Ruppitsch, K. Krziwanek, S. Metz-Gercek, and H. Mittermayer. *The Middle European Journal of Medicine,* 2009. 121: 86-90.

Summary: Discusses changes in MRSA over the past decade. Once known almost completely as a hospital pathogen, MRSA is now emerging in the community in persons without hospital-related risk factors. Recent evidence also has shown a link between livestock colonization and MRSA infections in persons working with these animals. Identifies three potential transmission routes of MRSA: from animal origin into the population; human-to-human contact from farm workers to the community; via food or by environmental contamination.

Methicillin-resistant *Staphylococcus aureus* (MRSA) strain ST398 is present in Midwestern swine and swine workers. T.C. Smith, M.J. Male, A.L. Harper, J.S. Kroeger, G.P. Tinkler, E.D. Mortiz, A.W. Capuano, L.A. Herwaldt, and D.J. Diekema. *PLoS One*. 2009. 4(1).

Summary: Establishes evidence of methicillin-resistant *Staphylococcus aureus* (MRSA) ST398 in swine and humans in the U.S. Swine and humans from two conventional swine production systems in Iowa and Illinois participated. MRSA was found in swine and humans working in close contact with the swine on one of the swine operations, the other operation had no MRSA-positive swine or employees. On the operation where MRSA was isolated, an inverse association between MRSA colonization of the nostrils of swine and age was observed with swine 15 weeks old or younger having higher odds of MRSA colonization than adult swine. Of 14 participating employees in close contact with swine, 9 were found to carry MRSA. Risk factors including age, gender, use of tobacco products, use of antimicrobial agents in the prior 3 months, having MRSA in the prior 12 months, and duration of employment were not associated with MRSA colonization. All MRSA isolated was confirmed to be ST398 and negative for the Panton-

and the state of Connecticut, the authors estimate that in 2005 more than 94,000 cases of such infections occurred, 18,650 of which were fatal.

Emergence of methicillin-resistant *Staphylococcus aureus* of animal origin in humans. I. van Loo, X. Huijsdens, E. Tuemersma, A. de Neeling, N. van de Sande-Bruinsma, D. Beaujean, A. Voss, and J. Kluytmans. *Emerging Infectious Diseases*, 2007. 13(12):1834-1839.

Summary: Reports that a new type of MRSA from an animal reservoir (pigs in the Netherlands) has recently entered the human population and is now responsible for greater than 20 percent of all MRSA in the Netherlands. As most nontypable MRSA isolates are resistant to doxycycline, the spread of MRSA may be facilitated by the abundant use of tetracyclines in pig and cattle farming.

Methicillin-resistant *Staphylococcus aureus* ST398 in humans and animals, Central Europe. W. Witte, B. Strommenger, C. Stanek, and C. Cuny. *Emerging Infectious Diseases*, 2007. 13(2): 255-258.

Summary: Studies recent human colonization by MRSA ST398, which in previous years had not been seen in humans. Animal-to-human transmission may occur with this strain; for example, a dog being treated for a wound infection transmitted ST398 to the staff of the veterinary practice where the dog was treated. Concludes that "MRSA exhibiting ST398 may colonize and cause infections in humans and in certain animal species such as dogs, horses and pigs."

Methicillin-resistant *Staphylococcus aureus* colonization in pigs and pig farmers. T. Khanna, R. Friendship, D. Dewey, and J.S. Weese. *Veterinary Microbiology,* 2008. 128:298-303.

Summary: This study, the first of MRSA and pig farms in Canada, found that the prevalence of MRSA colonization on pig farms was 45 percent; prevalence in pig farmers was 20 percent. Humans residing on farms where pigs were free of MRSA also tested negative for MRSA. The authors note another study in which MRSA was identified in food products intended for human consumption, but none originated in pigs. This study adds support to the hypothesis that MRSA can be transmitted between pigs and humans.

Pigs as source of methicillin-resistant *Staphylococcus aureus* CC398 infections in humans, Denmark. H.C. Lewis, K. Molbak, C. Reese, F.M. Aarestrup, M. Selchau, M. Sorum, and R.L. Skov. *Emerging Infectious Diseases,* 2008. 14(9): 1383-1389.

Summary: Provides evidence that persons exposed to animals on farms in Denmark, particularly pig farms, have an increased chance of being colonized or infected with MRSA CC398.

Methicillin-resistant and -susceptible *Staphylococcus aureus* sequence type 398 in pigs and humans. A.van Belkum, D.C. Melles, J.K. Peeters, W.B. van Leeuwen, E. van Duijkeren, X.W. Huijsdens, E. Spalburg, A.J. de Neeling, and H.A. Verbrugh. *Emerging Infectious Diseases*, 2008. 14(3):479-483.

Summary: Reports that MRSA ST398, primarily a pathogen of pigs, appears to be quite virulent and can cause bacteremia in humans. States that if MRSA ST398 obtains this pathogenicity, care should be taken not to introduce this strain into humans.

Transmission of methicillin-resistant *Staphylococcus aureus* strains between different kinds of pig farms. E. van Duijkeren, R. Ikawaty, M.J. Broekhuizen-Stins, M.D. Jansen, E.C. Spalburg, A.J. de Neeling, J.G. Allaart, A.van Nes, J.A.Wagenaar, and A.C. Fluit. *Veterinary Microbiology,* 2008. 126: 383-389.

MRSA

The impacts of methicillin-resistant Staphylococcus aureus *(MRSA) on certain areas across the country, veterinarians, health care employees and farmers.*

An outbreak of community-acquired foodborne illness caused by Methicillin-resistant *Staphylococcus aureus*. T.F. Jones, M.E. Kellum, S.S. Porter, M. Bell, and W. Schaffner. *Emerging Infectious Diseases.* 2002. 8(1): 82-84.

Summary: Describes an outbreak of acute gastroenteritis caused by methicillin-resistant *Staphylococcus aureus* (MRSA). Three family members consumed coleslaw and barbeque pork purchased from a market-delicatessen and after consuming the products experienced nausea, vomiting, and stomach cramps. Two of the three individuals sought treatment at a hospital. Indistinguishable MRSA isolates were found in a nasal swab from one asymptomatic food preparer at the market-delicatessen, a sample from the coleslaw, and stool cultures from the three patients. This is the first report of gastroenteritis due to MRSA however, *S. aureus* is a common cause of gastrointestinal illness in the US and therefore MRSA may play a larger role than previously thought.

Methicillin-resistant *Staphylococcus aureus* in pig farming. A. Voss, F. Loeffen, J. Bakker, C. Klaassen, and M. Wulf. *Emerging Infectious Diseases*, 2005. 11(12): 1965-1966.

Summary: Examines cases of MRSA colonization resulting from farmers' contact with pigs, how it moved though their families and was transmitted between a hospital patient and nurse. Reports that the frequency of MRSA among the group of regional pig farmers is more than 760 times higher than that among the general Dutch population.

Methicillin-resistant *Staphylococcus aureus* colonization in veterinary personnel. B.A. Hanselman, S.A. Kruth, J. Rousseau, D.E. Low, B.A. Willey, A. McGeer, and J.S. Weese. *Emerging Infectious Diseases*, 2006. 12(12): 1933-1938.

Summary: Reports a comprehensive evaluation of veterinary personnel for carriage of MRSA. Samples were taken from participants who resided in 19 different countries and rates of colonization were determined. Of the volunteers, 6.5 percent were positive for MRSA; those working with larger animals showed higher carriage rates (15.6 percent).

Hospitalizations and deaths caused by methicillin-resistant *Staphylococcus* aureus, United States, 1999–2005. E. Klein, D.L. Smith, and R. Laxminarayan. *Emerging Infectious Diseases,* 2007. 13(12): 1840-1846.

Summary: Reports on trends in MRSA infections between 1999 and 2005. The estimated rise in hospitalizations due to *Staphylococcus aureus* infections during this time was 62 percent, while the rate of MRSA infections more than doubled.

Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. R.M. Klevens, M.A. Morrison, J. Nadle, S. Petit, K. Gershman, S. Ray, L.H. Harisson, R. Lynfield, G. Dumyati, J.M. Townes, A.S. Craig, E.R. Zell, G.E. Fosheim, L.K. McDougal, R.B. Carey, and S.K. Fridkin. *Journal of the American Medical Association*, 2007. 285(15):1763-1771.

Summary: Finds that MRSA affects certain populations disproportionately, particularly African Americans. After researching invasive MRSA infections reported in hospitals in eight U.S. cities

Summary: Builds on previous work examining the similarities between extended-spectrum β-lactamase-producing *Escherichia coli* (ESBL-EC) isolated from chicken meat and human diagnostic specimens in the Netherlands. Multilocus sequence typing (MLST) revealed that 51 percent (22/43) of isolates from human rectal samples and 27 percent (4/15) of isolates from human blood cultures were the same as or related to ESBL-EC isolates from chicken meat. When the genetic composition of *E. coli* isolates from the three sources were compared, there was substantial overlap, especially between isolates from chicken meat and those from human rectal samples (representing the bacteria in the human gut). The similarities among ESBL-EC isolated from humans and chicken illustrates that food, specifically chicken meat, may contribute to the emergence of ESBL-EC infections among humans, raising questions about the use of antimicrobials and the presence of antimicrobial resistant bacteria among food animals.

antimicrobial agent. Additionally, 52 percent of all *S. aureus* were classified as multidrug resistant, meaning resistant to 3 or more antimicrobial classes. Samples showed resistance to several clinically important antibiotics including ciprofloxacin, quinupristin/dalfopristin, clindamycin, erythromycin, oxacillin, and daptomycin.

Identification and antimicrobial resistance of extraintestinal pathogenic *Escherichia coli* from retail meats. X. Xia, J. Meng, S. Zhao, S. Bodeis-Jones, S.A. Gaines, S.L. Ayers, and P.E. McDermott. *Journal of Food Protection.* 2011. 74(1). 38-44.

Summary: Extraintestinal *Eschericia coli* (ExPEC) is a type of *E. coli* that can cause infections outside of the intestines including urinary tract infections, meningitis, and wound infections. This study determines the prevalence of ExPEC in retail meat purchased in Georgia, Maryland, Oregon, and Tennessee in 2006. Reports 16 percent of 1,275 *E. coli*-positive samples were found to be ExPEC. The authors provide information on the distribution of serotypes and virulence genes found among positive samples. Concludes that many of the strains of ExPEC found in meat samples were of strains that can cause disease in humans and 80 percent were shown to be resistant to at least one antimicrobial agent, many of which are clinically relevant.

Prevalence of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) on retail meat in Iowa. B.M. Hanson, A.E. Dressler, A.L. Harper, R.P. Scheibel, S.E. Wardyn, L.K. Roberts, J.S. Kroeger, and T.C. Smith. *Journal of Infection and Public Health.* 2011. 4: 169-174.

Summary: Investigates the prevalence and types of *Staphylococcus aureus* present on retail pork, chicken, beef, and turkey products purchased at urban and rural stores across Iowa. Turkey demonstrated the highest prevalence of *S. aureus* with the bacteria found in 7/36 samples (19 percent). Turkey was followed by pork with 10/55 samples (18 percent), chicken with 8/45 samples (18 percent), and beef with 2/29 samples (7 percent) positive for *S. aureus*. Methicillin-resistant *S. aureus* (MRSA) was present in 2/55 pork samples (4 percent) only. Based on molecular methods, 7/27 *S. aureus* positive isolates (26 percent) were identified as ST398 and 4/27 were ST9, both livestock associated types. The two ST8 isolates also carried the Panton-Valentine Leukocidin virulence gene. Based on antibiotic susceptibility testing 5/27 isolates (19 percent) were susceptible to all antibiotics, 21/27 (78 percent) were resistant to penicillin, 18/27 (67 percent) were resistant to tetracycline, 6/27 were resistant to clindamycin (22 percent), 4/27 (15 percent) were resistant to erythromycin, and 2/27 (7 percent) were resistant to oxacillin. This study was focused on the potential for human contact with *S. aureus* and MRSA through contact with retail meat. For this reason, laboratory methods focused on the presence of these bacteria on the surface of retail meat and did not use more destructive laboratory methods that measure the prevalence of the bacteria anywhere in the meat samples as many other studies have done. Authors conclude that although this study showed lower prevalence of *S. aureus* and MRSA in retail meats than other studies, it shows that *S. aureus* and MRSA are present on the surface of retail meat and may be a source of human exposure.

Extended-spectrum β-Lactamase-producing *Escherichia coli* from retail chicken meat and humans: Comparison of strains, plasmids, resistance genes, and virulence factors. J.A.J.W. Kluytmans, I.T.M.A. Overdevest, I. Willemsen, M.F.Q. Kluytmans-van den Bergh, K. van der Zwaluw, M. Heck, M. Rijnsburger, C.M.J.E. Vandenbroucke-Grauls, P.H.M. Savelkoul, B.D. Johnston, D. Gordon, J.R. Johnson. *Clinical Infectious Diseases.* 2013. 56: 478-487.

Summary: Salmonella isolates from retail chicken were collected in central Pennsylvania from 2006-2007. Overall prevalence rates of Salmonella were 22.2 percent for a combination of open-air market samples, pre-packaged, organic and raised antibiotic free. Prevalence rates were not significantly different between these groups. These isolates were characterized by pulsed-field gel electrophoresis (PFGE) and compared to PulseNet data collected up to 2008. One collected poultry isolate matched directly to a human isolate that was acquired from a 17-year-old Philadelphia resident. The two isolates were collected within five months from each other and poultry consumption was listed as a possible risk factor suggesting that disease was likely caused from contaminated poultry.

Characterization of toxin genes and antimicrobial susceptibility of *Staphylococcus aureus* isolates from Louisiana retail meats. S. Pu, F. Wang, and B. Ge. *Foodborne Pathogens and Disease,* 2010. 1-8.

Summary: This study focuses on *Staphylococcus aureus* collected from retail meats in Louisiana. Isolates characterized included 152 *S. aureus* isolates, with 22 MRSA, for prevalence of 9 enterotoxin and 4 exotoxin genes as well as susceptibility profiles to 20 antimicrobials. Researchers found 85 percent were positive for at least one enterotoxin gene and 66 percent contained 2 to 4 enterotoxin genes. Staphylococcal enterotoxins cause approximately 185,000 food poisoning illnesses annually and occur upon ingestion of the carrier strains with symptoms such as vomiting, nausea, abdominal cramps and diarrhea. Antibiotic resistance was seen most often to penicillin (71 percent), ampicillin (68 percent) and tetracycline (67 percent). Erythromycin resistance (30 percent) and clindamycin resistance(18 percent) were also observed. Multidrug resistance was common in MRSA isolates and those samples from pork. The authors conclude that stringent food safety practice is needed for people who handle raw meat products to prevent food borne infections due to *S. aureus* contamination.

Antimicrobial susceptibility of *Staphylococcus aureus* from retail ground meats. A. Kelman, Y. Soong, N. Dupuy, D. Shafer, W. Richbourg, K. Johnson, T. Brown, E. Kestler, Y. Li, J. Zheng, P. McDermott, and J. Meng. *Journal of Food Protection*, 2011. 24(10). 1625-1629.

Summary: Retail ground beef, pork, and turkey were purchased at grocery stores in the Washington, D.C. area between March and August 2008. Reports 56 percent of 196 ground turkey samples, 28 percent of 198 ground beef samples, and 12 percent of 300 ground pork samples were positive for *Staphylococcus aureus*. Information on resistance to 22 antimicrobial agents is provided. All *S. aureus* from ground turkey, 89 percent from ground pork, and 11 percent from ground beef were resistant to at least one antimicrobial agent. More than half of the *S. aureus* found was resistant to tetracycline. One sample was positive for methicillin-resistant *S. aureus* (MRSA). Concludes that *S. aureus* in retail ground meats is not uncommon and many of these bacteria are resistant to at least one antimicrobial agent.

Multidrug-resistant *Staphylococcus aureus* in U.S. meat and poultry. A.E. Waters, T. Contente-Cuomo, J. Buchhagen, C.M. Liu, L. Watson, K. Pearce, J.T. Foster, J. Bowers, E.M. Driebe, D.M. Engelthaler, P.S. Keim, and L.B. Price. *Clinical Infectious Diseases,* 2011. 52(10). 1227-30.

Summary: Examines the presence and antibiotic-resistance patterns of *Staphylococcus aureus* in meat and poultry products in the U.S. Shows that 77 percent of 26 turkey products, 42 percent of 26 pork products, 41 percent of chicken products, and 37 percent of 38 beef products sampled were positive for *S. aureus.* An evaluation of resistance to 17 antimicrobials is provided and shows that 96 percent of the *S. aureus* positive-samples demonstrated resistance to at least one

higher virulence scores than beef and pork samples. These results support the hypothesis that antimicrobial-resistant *E. coli* in retail meats emerge from a host species-specific lineage due to the direct effect of selection pressure from use of antimicrobials or as part of the organisms' adaptations to their respective hosts.

Transient intestinal carriage after ingestion of antibiotic-resistant *Enterococcus faecium* from chicken and pork. T.L. Sorensen, M. Blom, D.L. Monnet, N. Frimodt-Moller, R.L. Poulsen, and F. Espersen. *New England Journal of Medicine,* 2009. 345(16): 1161-1166.

Summary: Reports on a study designed to test the ability of *Enterococci* from various meat sources to have sustained viability in the human intestine. Twelve volunteers ingested a suspension of *Enterococci* that originated from either a pig or chicken source that was resistant to at least one antibiotic. None of the 12 volunteers was colonized with resistant *Enterococci* at the onset of the experiment; however, eight of the 12 had antibiotic-resistant *Enterococci* isolated at six days following ingestion, and one had resistant *Enterococci* at 14 days' post ingestion. Concludes that ingestion of resistant *Enterococci* of animal origin leads to detectable concentrations of the same resistant strain in stools for up to 14 days.

Isolation and characterization of Methicillin-resistant *Staphylococcus aureus* strains from Louisiana retail meats. S. Pu, F. Han, and B. Ge. *Applied and Environmental Microbiology*. 2009. 75(1): 265-267.

Summary: Examines the presence of *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) in raw pork and beef products purchased from retail grocery stores consisting of seven supermarket chains in Baton Rouge, LA. Out of 90 pork samples, 41 were positive for *S. aureus* and 5 of those were found to be MRSA providing a prevalence of MRSA in pork of 6 percent. For beef products, 6/30 samples were positive for *S. aureus* and among those 1 was found to be MRSA for a prevalence of MRSA in beef products of 3 percent. Two of the six MRSA-positive pork samples contained the Panton-Valentine Leukocidin gene, which is considered the main virulence factor associated with skin and soft tissue infections.

Methicillin-resistant *Staphylococcus aureus* in food products: Cause for concern or complacency? J. A. J. W. Kluytmans. Clinical Microbiology and Infection, 2010. 16(1): 11-15.

Summary: A review on an emerging sequence type of MRSA ST398, which has been isolated from various food animals. A recent study in the U.S. observed a contamination rate of 39.2 percent for *S. aureus* on retail meats and in that group 5 percent was MRSA. Studies abroad have shown rates of MRSA contaminating retail meats as high as 11.9 percent. The author suggests that even though ST398 does not appear to spread easily among humans this assumption needs to be confirmed in well-designed studies. The spread of ST398 from animals to humans needs to be monitored as the potential threat from the retail food reservoir has widespread potential implications on human health.

Multidrug-resistant *Salmonella* isolates from retail chicken meat compared with human clinical isolates. N.M. M'ikanatha, C.H. Sandt, A.R. Localio, D. Tewari, S.C. Rankin, J.M. Whichard, S.F. Altekruse, E. Lautenbach, J.P. Folster, A. Russo, T.M. Chiller, S.M. Reynolds, and P.F. McDermott. *Foodborne Pathogens and Disease,* 2010. 7:8 929-934.

antimicrobial resistance. Antibiotic-free labeling was identified as a risk factor for contamination. ExPEC was found in 4 percent of miscellaneous food, 19 percent of pork, and 46 percent of poultry samples and resistance to at least one antibiotic was present in 27, 85, and 94 percent, respectively. Four of the ExPEC positive isolates from food closely resembled positive isolates from humans. Concludes that retail foods are often contaminated with antibiotic-resistant *E. coli* and ExPEC *E. coli* is also present. Food may serve as an important vehicle for the transmission of antibiotic resistant ExPEC bacteria.

Contamination of retail foods, particularly turkey, from community markets (Minnesota, 1999-2000) with antimicrobial-resistant and extraintestinal pathogenic *Escherichia coli*. J.R. Johnson, P. Delavari, T.T. O'Bryan, K.E. Smith, and S. Tatini. *Foodborne Pathogens and Disease*. 2005. 2(1):38-49.

Summary: Provides results from a one-year retail market survey of the prevalence of antimicrobial-resistant *Escherichia coli* in a range of retail foods. Food products were purchased systematically from 16 retail markets, including large economy and luxury chains, small economy chains, locally owned cooperatives, and farmer's markets (only during the summer). Sixteen percent (35/222) of vegetables sampled were positive for *E. coli*, as well as 5 percent (4/74) of fruit items, 100 percent (10/10) beef, (3/3) pork, (28/28) turkey products, and 89 percent (8/9) chicken products. Ten extraintestinal pathogenic *E. coli* (ExPEC) positive samples containing a range of virulence genes were found in turkey products that differed by type, store, and purchase date. ExPEC isolates were missing several virulence genes commonly found in human clinical *E. coli* isolates; however, four of the ExPEC isolates had virulence profiles, phylogenetic backgrounds, and O antigens that resembled clonal groups associated with human infection. Susceptibility testing for 12 antimicrobial agents demonstrated that resistance to tetracycline and sulfisoxazole was most common, followed by resistance to ampicillin, cefazolin, and gentamicin. No resistance to nitrofurantoin or ciprofloxacin was identified. Resistance was more common in meat products than other products and isolates resistant to more than 4 drugs were recovered from one beef item and 29 percent of turkey items. Suggests meat is more frequently contaminated with *E. coli* and meat-source and produce-source *E. coli* differs in virulence, phylogenetic background, and antimicrobial resistance, and therefore in public health implications. Additionally, turkey may be an important source of human exposure to resistant and potentially pathogenic *E. coli* including ExPEC.

Resistance in bacteria of the food chain: Epidemiology and control strategies. F.M. Aarestrup, H.C. Wegener, and P. Collignon. *Expert Reviews,* 2008. 6(5): 733-750.

Summary: Reviews bacterial resistance due to the use of antimicrobials in food animals and their transferability to humans in the form of pathogens. States that limiting the selective pressure in food animal production, especially those antibiotics that are critically important to human health, will help control the emergence of resistant bacteria most efficiently.

Molecular analysis of *Escherichia coli* from retail meats (2002–2004) from the United States National Antimicrobial Resistance Monitoring System. J.R. Johnson, J.S. McCabe, D.G. White, B. Johnston, M.A. Kuskowski, and P. McDermott. *Clinical Infectious Diseases,* 2009. 49: 195-201.

Summary: Researchers screened 287 *E. coli* isolates collected by the National Antimicrobial Resistance Monitoring System (NARMS) for virulence-associated genes. Resistant and susceptible strains differed minimally based on the assessed virulence factors; however, the four meat types screened showed a great variance as chicken and turkey isolates had consistently

Concurrent quantitation of total *Campylobacter* and total ciprofloxacin-resistant *Campylobacter* loads in rinses from retail raw chicken carcasses from 2001 to 2003 by direct plating at 42 degrees Celsius. R. Nannapaneni, R. Story, K.C. Wiggins, and M.G. Johnson. *Applied and Environmental Microbiology*, 2005. 71(8): 4510-4515.

Summary: Analyzes the total amount of *Campylobacter* present in retail chicken as well as in ciprofloxacin-resistant isolates. Finds that ciprofloxacin-resistant *Campylobacter* persisted throughout the two-and-a-half-year study, showing a reservoir of resistance in the U.S. food market.

Sulfamethazine uptake by plants from a manure-amended soil. H. Dolliver, K. Kumar, and S. Gupta. *Journal of Environmental Quality*, 2007. 36:1224-1230.

Summary: Studies the uptake of sulfamethazine, an antibiotic extensively used in animal agriculture for therapeutic and subtherapeutic purposes, in corn, lettuce and potatoes when manure-amended soil is used as the growing medium. Following 45 days of growth, all plants tested were contaminated with the antibiotic in varying concentrations.

Antimicrobial drug-resistant *Escherichia coli* from humans and poultry products, Minnesota and Wisconsin, 2002–2004. J.R. Johnson, M.R. Sannes, C. Croy, B. Johnston, C. Clabots, M.A. Kuskowski, J. Bender, K.E. Smith, P.L. Winokur, and E.A. Belongia. *Emerging Infectious Diseases*, 2007, 13(6): 838-846.

Summary: Studies susceptible and resistant *E. coli* collected from hospital patients, healthy vegetarians and poultry that were raised conventionally and without antibiotics. Suggests that many resistant human isolates may originate from poultry. Isolates from healthy vegetarians also follow this pattern, suggesting that avoidance of poultry consumption does not decrease the possibility of carrying drug-resistant *E. coli* from poultry.

The isolation of antibiotic-resistant *Salmonella* from retail ground meats. D.G. White, S. Zhao, R. Sudler, S. Ayers, S, Friedman, S. Chen, P.F. McDermott, S. McDermott, D.D. Wagner, and J. Meng. *New England Journal of Medicine*, 2007. 345(16):1147-1154.

Summary: Researchers tested *Salmonella* from samples of ground chicken, pork, beef and turkey purchased at three supermarkets in the Washington, DC, area. Of 200 samples, 41 (20 percent) contained *Salmonella*. Eighty-four percent of those were resistant to at least one antibiotic and 53 percent were resistant to at least three antibiotics. Sixteen percent were resistant to ceftriaxone, the drug of choice for treating salmonellosis in children.

Antimicrobial-resistant and extraintestinal pathogenic *Escherichia coli* in retail foods. J.R. Johnson, M.A. Kuskowski, K. Smith, T.T. O'Bryan, and S. Tatini. *Journal of Infectious Diseases*. 2005. 191:1040-1049.

Summary: Presents results from a two-year survey of the presence of antimicrobial-resistant *Escherichia coli*, and specifically, extraintestinal pathogenic *E. coli* (ExPEC), in a sampling of foods. To determine differences between retail markets and food types, samples of meat and other foods were systematically taken on a weekly basis from a range of retail markets based on a pre-determined schedule. Approximately 24 percent (396/1648) of samples were positive for *E. coli* with proportions varying by food type (miscellaneous foods: 9 percent; pork: 69 percent; poultry: 92 percent). Among beef and pork being ground was a risk factor for the presence of *E. coli* while natural-store source was associated with a reduction in the presence of the bacteria and

RETAIL PRODUCTS

How industrial food animal production affects the food supply.

An evaluation of methods to assess the effect of antimicrobial residues on the human gut flora. D. Corpet. *Veterinary Microbiology,* 1993. 35(3-4):199-212.

Summary: Reviews the effects of antimicrobial residues on the human gut flora and concludes that "most resistant enterobacteria in the human gut of untreated people come from bacterial contamination of raw foods." This assumption stems from a study previously completed by the author in which a sterile diet was given to seven healthy volunteers with an outcome of reduced antibiotic-resistant bacteria in stools.

Quinoline-resistant *Campylobacter jejuni* infections in Minnesota, 1992–1998. K.E. Smith, J.M. Besser, C.W. Hedberg, F.T. Leano, J.B. Bender, J.H. Wickland, B.P. Johnson, K.A. Moore, and M.T. Osterholm. *New England Journal of Medicine,* 1999. 340(20):1525-1532.

Summary: Reports that ciprofloxacin-resistant *C. jejuni* was isolated from 14 percent of 91 domestic chicken products obtained from retail markets in 1997. The number of quinolone-resistant infections acquired domestically has increased, largely because of the acquisition of resistant strains from poultry. Resulting infections may require additional antimicrobial therapy, as fluoroquinolones such as ciprofloxacin are commonly prescribed for diarrheal illnesses caused by *Campylobacter jejuni.*

Isolation of antimicrobial-resistant *Escherichia coli* from retail meats purchased in Greater Washington, DC, USA. C.M. Schroeder, D.G. White, B. Ge, Y. Zhang, P.F. McDermott, S. Ayers, S. Zhao, and J. Meng. International Journal of Food Microbiology, 2003. 85: 197-202.

Summary: Retail meat samples were collected and analyzed from the DC area for presence of *E. coli.* Data on resistance to 11 antimicrobials are given with a large portion showing resistance to such antibiotics as tetracycline (59 percent), sulfamethoxazole (45 percent), streptomycin (44 percent), ampicillin (35 percent) and gentamicin (12 percent). The authors conclude that their findings suggest retail meats may often be contaminated with resistant *E. coli.*

The incidence of antimicrobial-resistant *Salmonella* spp. on freshly processed poultry from US Midwestern processing plants C.M. Logue, J.S. Sherwood, P.A. Olah, L.M. Elijah, and M.R. Dockter. *Journal of Applied Microbiology,* 2003. 94: 16-24

Summary: A study to determine the occurrence of antimicrobial-resistant *Salmonella* spp. on processed turkey at poultry plans in the Midwestern U.S. Samples (surface swabs from carcasses: pre- and post chill and chill water from tanks) were taken from two plants at monthly intervals for one year. Overall incidence of *Salmonella* was around 16.7 percent, with a greater percentage of the pathogen observed on carcasses both pre- and post-chill, with post-chill showing decreased occurrence compared to pre-chill. *Salmonella* from the study had varying levels of antimicrobial resistance. The most common resistance was seen to tetracycline, streptomycin, sulfamethaxozole and ampicillin. Chlorination of chill water is thought to cause this reduction in contamination; however, the authors state that infections would be difficult to treat in the future if chlorine resistance is a factor in promoting selection of bacteria that have other resistance mechanisms.

STAs had no statistically significant impact on production given other inputs. The estimates indicate that growers and integrators can adapt to STA suspensions without declines in production.

conventional operations were resistant to two or more antimicrobial agents. The study demonstrates that within one poultry company in North Carolina, the prevalence of *Salmonella* and antibiotic-resistant *Salmonella* was greater on conventional operations as compared to organic operations. However, as antibiotic resistance was found in both operations this may signal circulation of organisms within a company's farms.

Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. M.A. Leverstein-van Hall, C.M. Dierikx, J. Cohen Stuart, G.M. Voets, M.P. van den Munckhof, A. van Essen-Zandbergen, T. Platteel, A.C. Fluit, N. van de Sande-Bruinsma, J. Scharinga, M.J.M.Bonten, and D.J. Mevius. *Clinical Microbiology and Infection.* 2011, 17(6):873-880.

Summary: An increase in infections caused by Gram-negative bacteria producing extended spectrum beta-lactamases (ESBL) has been observed globally. In The Netherlands, there is low human use of antibiotics but higher levels of use in poultry production. This study examined the distribution of ESBL genes, plasmids, and strain genotypes in *Escherichia coli* found in retail chicken in 2006, in poultry in 2010, and determined the distribution of isolates from Dutch patients with "poultry associated" (PA) ESBL genes, plasmids, and strains in 2009. Of 98 samples from chicken retail meat, 94 percent contained at least one isolate thought to be positive for ESBL. Of 409 ESBL-positive *E. coli* isolates from humans, 35 percent contained ESBL genes and 19 percent had ESBL genes located on plasmids that were indistinguishable from those found in poultry isolates. The most common genes found in human isolates, $bla_{CTX\text{-}M\text{-}1}$ and $bla_{TEM\text{-}52}$, were also the most common genes found in poultry and retail chicken meat and 39 percent of ESBL-producing *E. coli* found in retail meat belonged to genotypes also found in humans. Study results suggest transmission of ESBL-producing *E. coli* between poultry and humans, potentially through contact with retail chicken; however, due to study limitations, further research is required.

Prevalence of types of methicillin-resistant *Staphylococcus aureus* in turkey flocks and personnel attending the animals. A. Richter, R. Sting, C. Popp, J. Rau, B.A. Tenhagen, B. Guerra, H.M. Hafez, A. Fetsch. *Epidemiology and Infection.* 2012. 140(12): 2223-2232.

Summary: The prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) among turkeys was 143/200 turkeys (72 percent) from 90 percent of the 20 flocks examined. Among 59 people sampled from 12 farms, 22 (37 percent) were positive for MRSA carriage. Thirteen people tested were found to carry the same type of MRSA as that isolated from the animals or in the environment from the operation, while five carried a different type. Increased contact with turkeys per week increased the odds of MRSA carriage. Almost all MRSA isolates found in this study were also resistant to tetracycline (98 percent) and large proportions were resistant to clindamycin and erythromycin (83 percent), kanamycin (42 percent), gentamicin (27 percent) and ciprofloxacin (31 percent).

Foregoing Sub-therapeutic Antibiotics: the Impact on Broiler Grow-out Operations. J.M. MacDonald and S.-L. Wang. Applied Economic Perspectives and Policy (2011): 1-20. Advance Access published January 6, 2011. doi:10.1093/aepp/ppq030

Summary: Data from a national survey of broiler to analyze the use of subtherapeutic antibiotics (STAs) among broiler growers. About 55% of farms may use STAs. Those who don't use STAs clean out their barns more consistently and use all-in all-out production, feed an all-vegetable diet, follow some sort of animal welfare guidelines, have newer houses, and are also more likely to have tunnel ventilation and evaporative cooling in their houses. Producers who do not use

Daignault, A. Desruisseau, W. Demczuk, L. Hoang, G.B. Horsman, J. Ismail, F. Jamieson, A. Maki, A. Pacagnella, and D.R. Pillai. *Emerging Infectious Diseases*, 2010. 16(1): 48-54.

Summary: Studies *Salmonella* Heidelberg, a frequently reported cause of infections in North America with sources linked to consumption of poultry, eggs or egg-containing products. Compares resistance rates of *Salmonella* Heidelberg isolates collected from retail chicken to ceftiofur, a third-generation cephalosporin, with rates of human infections that also were resistant to ceftiofur during a period from 2003 to 2008. During this time frame ceftiofur was removed from extralabel use in chicken hatcheries in Québec, resulting in a dramatic decrease in ceftiofur resistance in *Salmonella* Heidelberg and *E. coli* in retail chicken. A similar decrease is shown in resistant human infections of *Salmonella* Heidelberg. Suggests that managing ceftiofur use at the hatchery level may control resistance rates to extended-spectrum cephalosporins. A partial reintroduction of ceftiofur use in hatcheries in 2007 caused a rise in ceftiofur resistance in *E. coli,* but at lower levels than those seen in 2003 to 2004.

Veterinary pharmaceuticals and antibiotic resistance of Escherichia coli isolates in poultry litter from commercial farms and controlled feeding trials. V. Furtula, E.G. Farrell, F. Diarrassouba, H. Rempel, J. Pritchard, and M.S. Diarra. *Poultry Science*, 2010. 89:180-188.

Summary: This study found that there were antimicrobial residues in broiler litter from both a controlled environment, where chickens were fed a diet of feed with additives of bacitracin, chlortetracycline, monensin, narasin, nicarbazin, penicillin, salinomycin and virginiamycin and from commercial farms where the same feed additives were also used. Antimicrobials are not fully absorbed by animals in some cases and will be excreted into the litter leaving a residue of antibiotics that may then be applied to soil for crop fertilization. If application occurs, soil microbes will be subjected to these antibiotic pressures and may develop resistance themselves. There is also evidence for plants to uptake antimicrobial agents and can become a source of exposure to such compounds. *E. coli* isolates were collected from poultry litter from commercial farms and were found to be resistant to at least seven different antibiotics. Isolates from commercial farms showed a higher rate of resistance possibly due to the frequent use of feeds that are available with multiple antibiotics incorporated causing increased resistance. Resistance to such antibiotics as trimethoprim-sulfamethoxazole from isolates collected on commercial farms is of concern as this is a leading treatment of urinary tract infections.

Prevalence and distribution of *Salmonella* in organic and conventional broiler poultry farms. W.Q. Alali, S. Thakur, R.D. Berghaus, M.P. Martin, and W.A. Gebreyes. *Foodborne Pathogens and Disease.* 2010. 7(11): 1363-1371.

Summary: Studies the prevalence of *Salmonella* and antimicrobial resistance on one company's USDA-certified organic broiler chicken operations and conventional broiler operations in North Carolina. Samples from fecal floor droppings, the feed hopper, feed lines, house main water line, and in-house drinking nipples were taken from three organic barns and four conventional barns. *Salmonella* was present in 13 of 300 samples (4 percent) taken from organic operations and 115 of 400 samples (29 percent) from conventional operations. Conventional operations demonstrated 11.9 times the prevalence odds of *Salmonella* in fecal samples as compared to organic operations. The prevalence odds ratio for *Salmonella* in feed samples from conventional vs. organic farms was 7.2. Approximately, 25 percent of isolates from organic operations and 1.7 percent of isolates from conventional operations were susceptible to all antimicrobial agents tested. Additionally, 41 percent (5/12) of isolates from organic operations and 62 percent (36/58) of isolates from

Relationships between multidrug-resistant *Salmonella enterica* Serovar Schwarzengrund and both broiler chickens and retail chicken meats in Japan. T. Asai, K. Murakami, M. Ozawa, R. Koike, and H. Ishikawa. *Japanese Journal of Infectious Diseases,* 2009. 62: 198-200.

Summary: A *Salmonella* strain that causes invasive salmonellosis in humans was isolated from broiler chickens and retail chicken meats in Japan. Numerous isolates showed multidrug resistance.

Fate of antimicrobial-resistant *Enterococci* and *Staphylococci* and resistance determinants in stored poultry litter. J.P. Graham, S.L. Evans, L.B. Price, and E.K. Silbergeld. *Environmental Research,* 2009. 109: 682-689.

Summary: Studies the storage of poultry litter and the stability of bacteria and resistance genes during storage. Finds that over a 120-day period, typical storage practices of poultry litter are not sufficient for eliminating drug-resistant *Enterocci* and *Staphylococci,* which may then be delivered to the environment by land application, aerosolization or water contamination during runoff.

Antibiotic-resistant *Enterococci* and *Staphylococci* isolated from flies collected near confined poultry feeding operations. J.P. Graham, L.B. Price, S.L. Evans, T.K. Graczyk, and E.K. Silbergeld. *Science of the Total Environment,* 2009. 407(8): 2701-2710.

Summary: Investigators collected poultry litter and trapped flies around poultry farms to determine the extent of bacteria present and their resistance-gene profile. Results suggest that flies around poultry operations harbor resistant bacteria in their digestive tracts and exterior surfaces. This could result in human exposure to resistant bacteria that arise from antimicrobial use on poultry farms. Highlights the persistence of resistant genes in the environment and the pool of resistance associated with the use of antibiotics in feed additives.

***Salmonella Heidelberg* Ceftiofur-related resistance in human and retail chicken isolates.** Public Health Agency of Canada. 2009.

Summary: In response to public health concerns about the rise of resistance in isolates of *Salmonella* and *E. coli* to ceftiofur, all broiler chicken hatcheries in Québec voluntarily stopped using ceftiofur in February 2005. This publication reports a decrease in the number of ceftiofur-resistant isolates in both chicken and human *S. heidelberg* isolates and in chicken *Escherichia coli* following the voluntary withdrawal of ceftiofur in hatching and day-old chicks in Québec.

Antibiotic resistance of *Escherichia Coli* isolated from poultry and poultry environment of Bangladesh. M.A. Akond, S.M.R. Hassan, S. Alam, and M. Shirin. *American Journal of Environmental Sciences*, 2009. 5 (1): 47-52.

Summary: A study of *E. coli* isolated from poultry sources in Bangladesh. Resistance was high to many antibiotics including: penicillin, streptomycin, kanamycin, ampicillin and erythromycin. Resistance was not seen to gentamicin. The authors state that the widespread use of antibiotics has lead to resistance development that can be transmitted to human pathogens; they suggest that excess use or abuse of antibiotics should be reduced or stopped to ensure public safety.

Ceftiofur resistance in *Salmonella enterica* Serovar Heidelberg from chicken meat and humans, Canada. L. Dutil, R. Irwin, R. Finley, L. King Ng, B. Avery, P. Boerlin, A. Bourgault, L. Cole, D.

Effect of macrolide usage on emergence of erythromycin-resistant *Campylobacter* isolates in chickens. J. Lin, M. Yan, O. Sahin, S. Pereira, Y. Chang, and Q. Zhang. *Antimicrobial Agents and Chemotherapy*. 2007. 51(5): 1678-1686.

Summary: Erythromycin is a macrolide antimicrobial often used to treat *Campylobacter* infection in humans. This article presents information from experiments conducted to determine the emergence of erythromycin-resistant (Ery^r) *Campylobacter jejuni* and *Campylobacter coli* under selection pressure of macrolide use in a laboratory setting. Further discusses mechanisms associated with resistance to erythromycin. Results are presented from three experiments examining treatment of chickens with water containing tylosin, a macrolide-class antibiotic, and two experiments examining treatment of chickens with feed containing tylosin. Experiments show that chickens receiving a three-day therapeutic dose of tylosin in water (0.53 g/liter) shed significantly less *C. jejuni* and *C. coli* during treatment, but when treatment ended, resumed shedding a similar amount of the organisms as the control group that did not receive treatment. No Ery^r mutants were found in the treatment or control group in these experiments. Effects of long-term exposure to tylosin are examined by two experiments one which chickens were inoculated with *C. jejuni* at 3 days of age and one at 17 days of age. Treatment groups in each experiment were given feed containing tylosin at a subtherapuetic dose used for growth promotion (50 mg/kg) and control groups were provided unmedicated feed. Both experiments showed an initial reduction in shedding of *C. jejuni* in the medicated group; however, by day 31 and 17 in the two experiments respectively, Ery^r mutants were observed in chickens receiving medicated feed. No Ery^r mutants were observed in the control group in either experiment. Concludes that *C. jejuni* and *C. coli* have low rates of spontaneous mutation to Ery^r when therapeutic dosing is used but extended use of a macrolide drug as a growth promoter resulted in the emergence of Ery^r *C. jejuni* under laboratory conditions. However, the results presented here may not be used to predict development of antibiotic resistance on poultry farms as many factors may differ between the laboratory and poultry farm setting.

Antimicrobial resistance of old and recent *Staphylococcus aureus* isolates from poultry: First detection of livestock-associated methicillin-resistant strain ST398. M. Nemati, K. Hermans, U. Lipinska, O. Denis, A. Deplano, M. Struelens, L.A. Devriese, F. Pasmans, and F. Haesebrouck. *Antimicrobial Agents and Chemotherapy*, 2008. Oct: 3817-3819.

Summary: Compares the resistance profiles of *Staphylococcus aureus* isolates collected from chickens in the 1970s with profiles from healthy chickens in 2006. Finds that resistant levels to eight of the drugs tested were significantly greater in the 2006 samples.

Food animal transport: A potential source of community exposures to health hazards from industrial farming (CAFOs). A.M. Rule, S.L. Evans, and E.K. Silbergeld. *Journal of Infection and Public Health*, 2008. 1(1): 33-39.

Summary: Compares air samples collected while cars with bacterial-collection equipment were driven behind poultry transport vehicles with background samples taken during normal driving conditions. Twenty-five percent of samples collected while following poultry transport vehicles were resistant at least one antimicrobial, while all background samples were susceptible. Suggests that open-air poultry transport vehicles may play a role in spreading resistant bacteria that originated from the administration of antimicrobials to food animals.

dalfopristin resistance gene and inducible quinupristin-dalfopristin resistance in human fecal *E. faecium*. The continued use of virginiamycin may increase the potential for streptogramin-resistant *E. faecium* infection in humans."

Subtherapeutic tylosin phosphate in broiler feed affects *Campylobacter* on carcasses during processing. M.E. Berrang, S.R. Ladely, R.J. Meinersmann, and P.J. Fedorka-Cray. *Poultry Science*, 2007. 86:1229-1233.

Summary: Studies cross-resistance of tylosin and erythromycin (both macrolide drugs). Erythromycin is often the drug of choice for treating campylobacteriosis, and tylosin is approved at subtherapeutic levels for use in broiler feed for growth promotion. Seventy chicks were divided into two groups, half raised on tylosin, half without. Carcasses of broilers fed tylosin had lower numbers of *Campylobacter*, but all the *Campylobacter* found were resistant to erythromycin. No *Campylobacter* isolated from the control carcasses were resistant. Concludes that application of tylosin phosphate in feed results in lower numbers of *Campylobacter*, but those that remain are resistant to erythromycin.

Growth promoting antibiotics in food animal production: An economic analysis. J.P. Graham, J.J. Boland, and E. Silbergeld. *Public Health Reports*, 2007. 122:79-87.

Summary: Examines the economic effect of removing antibiotics used for growth promotion in broiler chickens using data published by Perdue. Positive production changes were associated with use, but were insufficient to offset the cost of the antibiotics. The net effect of using growth-promoting antibiotics was a lost value of $.0093 per chicken (about 0.45 percent of total cost).

Development of macrolide-resistant *Campylobacter* in broilers administered subtherapeutic or therapeutic concentrations of tylosin. S.R. Ladely, M.A. Harrison, P.J. Fedorka-Cray, M.E. Berrang, M.D. Englen, and R.J. Meinersmann. *Journal of Food Protection*, 2007. 70(8):1915-1951.

Summary: Looks at the impact of antibiotic use on increasing the amount of resistant bacteria in an environment. Poultry were divided into groups of 25 birds: the treatment group was given either therapeutic or subtherapeutic doses of tylosin beginning at two weeks of age while the control group was isolated and not given any antimicrobials. The animals fed subtherapeutic and therapeutic doses of tylosin tested positive for resistant bacteria; no resistant strains were found among the birds that did not get treated with tylosin. The birds treated with subtherapeutic doses of tylosin also showed increased resistance compared with the birds treated with therapeutic doses.

Elevated risk of carrying gentamicin-resistant *Escherichia coli* among U.S. poultry workers. L.B. Price, J.P. Graham, L.G. Lackey, A. Roess, R. Vailes, and E. Silbergeld. *Environmental Health Perspectives*, 2007. 15(12):1738-1742.

Summary: Examines poultry workers and residents on the eastern shore of Maryland and Virginia. Poultry workers had 32 times the odds of being colonized with gentamicin-resistant *E. coli* as community residents; the poultry workers also had an elevated risk of carrying multidrug-resistant *E. coli*. Concludes that "occupational exposure to live animals in the broiler chicken industry may be an important route of entry for antimicrobial-resistant bacteria in to the community."

Summary: Poultry was withdrawn in Belgium in June 1999 after a contaminant was found in feed. According to a model designed from the sentinel surveillance system, *Campylobacter* infections decreased by 40 percent during that month—from 153 cases per week to 94 cases. States that by using the ban as an epidemiologic tool, the rate of *Campylobacter* infections attributable to poultry was determined to be greater than 40 percent.

The effect of withdrawing growth promoting antibiotics from broiler chickens: A long-term commercial industry study. H.M. Engster, D. Marvil, and B. Stewart-Brown. *The Journal of Applied Poultry Research,* 2002. 431-436.

Summary: A comprehensive study where removal of growth promoting antibiotics (GPA) from broiler chickens was compared with those still receiving GPA. Average reduction of livability was only 0.2 percent on the Delmarva Peninsula (DMV) and 0.14 percent in North Carolina (NC). However, fluctuations were noted in livability from a reduction of 0.5 percent to a positive impact on livability of 0.3 percent. The average reduction in body weight was 0.03 lb on DMV and 0.04 lb in NC but this decline did not start until after the first year of the trial. Feed conversion (weight of food/body weight gain) was not adversely affected in the study for either location. Removal of GPA also resulted in no reports of field outbreaks of disease and total farm condemnations were not affected.

Fluoroquinolone-resistant *Campylobacter* isolates from conventional and antibiotic-free chicken products. L.B. Price, E. Johnson, R. Vailes, and E. Silbergeld. *Environmental Health Perspectives,* 2005. 113: 557-560.

Summary: Concludes that there is no difference in *Campylobacter* contamination between conventionally raised chickens and poultry raised antibiotic-free; however, conventionally raised poultry is more likely to be resistant to antibiotics than chickens raised antibiotic-free. The findings also suggest that fluoroquinolone-resistant isolates of *Campylobacter* may persist after the usage of fluoroquinolones in poultry production has ceased.

Similarity between human and chicken *Escherichia coli* isolates in relation to ciprofloxacin resistance status. J.R. Johnson, M.A. Kuskowski, M. Menard, A. Gajewski, M. Xercavins, and J. Garau. *The Journal of Infectious Diseases,* 2006. 194(1): 71-78.

Summary: Studies the similarities of *E. coli* isolates collected from humans and chickens that were resistant to ciprofloxacin. Finds that resistant *E. coli* in humans appears to have a profile similar to that of resistant *E. coli* collected from chickens, suggesting that the use of antimicrobials in poultry production is leading to resistant *E. coli* that are being transferred to humans, possibly though contaminated meats.

Use of streptogramin growth promoters in poultry and isolation of streptogramin-resistant *Enterococcus faecium* from humans. A.L. Kieke, M.A. Borchardt, B.A. Kieke, S.K. Spencer, M.F. Vandermause, K.E. Smith, S.L. Jawahir, and E.A. Belongia. *The Journal of Infectious Diseases,* 2006. 194(9): 1200-1208.

Summary: Examines virginiamycin use in poultry and its effect on cross-resistance to quinupristin-dalfopristin, a drug also in the streptogramin category that is intended for treating vancomycin-resistant *Enterococcus faecium* infections in humans. The study enrolled patients from hospitals and vegetarians and compared the samples from humans with samples collected from retail poultry meats. Reports that "poultry exposure is associated with a quinupristin-

POULTRY

The effects of poultry production on farm workers, public health and the spread of antibiotic-resistant bacteria.

Direct transmission of *Escherichia coli* from poultry to humans. A.A. Ojenyiyi. *Epidemiology and Infection*, 1989. 103(3): 513-522.

Summary: Compares the resistance traits of *E.coli* collected from free-range poultry with those from poultry in a large-scale commercial facility. Reports that resistance to the antibiotics tested occurred only in those samples collected from birds in a commercial setting. Attendants from the commercial facilities also were found to contain resistant bacteria while samples from villagers in the community were negative. The authors also demonstrated that attendants contract bacteria from birds in their care by conducting a study where they infected birds with a known type of resistant *E. coli* and screened the attendants for the same bacteria.

Quinolone resistance in *Campylobacter* isolated from man and poultry following the introduction of fluoroquinolones in veterinary medicine. H.P. Endtz, G.J. Ruijs, B. van Klingeren, W.H. Jansen, T. van der Reyden, and R.P. Mouton. *The Journal of Antimicrobial Chemotherapy*, 1991. 27(2): 199-208.

Summary: Reports the results of tests for quinolone resistance in 883 strains of *Campylobacter* bacteria isolated between 1982 and 1989 from human stool and poultry products. *Campylobacter* isolated from poultry increased in resistance from 0 percent to 14 percent in that time, while resistance in human isolates rose from 0 percent to 11 percent. Results suggest that the increase is mainly due to use of enrofloxacin, a fluoroquinolone, in poultry.

High-frequency recovery of quinupristin-dalfopristin-resistant *Enterococcus faecium* isolates from the poultry-production environment. J.R. Hayes, A.C. McIntosh, S. Qaiumi, J.A. Johnson, L.L. English, L.E. Carr, D.D. Wagner, and S.W. Joseph. *Journal of Clinical Microbiology,* 2001. 39(6): 2298-2299.

Summary: Studies the extent of resistance to quinupristin-dalfopristin, a drug reserved for human use to treat vancomycin-resistant enterococci, in *Enterococcus faecium.* Finds that resistance to this antimicrobial ranged between 51 percent and 78 percent in isolates screened from the food-production environment.

Antibiotic resistance of faecal *Escherichia coli* in poultry, poultry farmers and poultry slaughterers. A.E. van den Bogaard, N. London, C. Driessen, and E.E. Stobberingh. *Journal of Antimicrobial Chemotherapy,* 2001. 47:763-771.

Summary: Reports a survey of *E. coli* in poultry and workers who were in close contact with animals. Finds that the highest resistance rates were in turkeys, closely followed by broilers. Isolates collected from the laying-hen population were much lower, possibly because of the infrequent use of antibiotics in these animals. In the human population the same results followed, with turkey workers' isolates showing greater resistance than those from broilers or laying-hens. Results also strongly suggest the transmission of resistant clones and resistance plasmids of *E. coli* from broilers and turkeys to humans.

The dioxin crisis as experiment to determine poultry-related *Campylobacter enteritis*. A. Vellinga and F. Van Loock. *Emerging Infectious Diseases,* 2002. 8(1): 19-22.

from pigs, the environment, and humans at the same farms. MRSA in this study had high levels of resistance to tetracyclines (chlortetracycline, oxytetracycline), neomycin, and spectinomycin with some differences in resistance profiles observed between STs. Following short-term exposure to MRSA-positive pork farms, MRSA may be easily shared between pigs and humans but may not become an established colonizer of humans.

A longitudinal study on persistence of antimicrobial resistant *Campylobacter* in distinct swine production systems at farm, slaughter, and environment. M.P. Quintana-Hayashi, S. Thakur. *Applied Environmental Microbiology*. 2012. 78(8):2698-705.

Summary: Reports on the prevalence and antibiotic resistance of *Campylobacter* found in antibiotic-free and conventional swine operations at the farm and at slaughter facilities. The prevalence of *Campylobacter* isolated from swine raised on antibiotic-free and conventional operations was similar. *Campylobacter* from conventionally raised swine had a greater prevalence of resistance to ciprofloxacin (17 vs. 1 percent), tetracycline (88 vs. 48 percent), and naladixic acid (1 vs. 17 percent) as compared to antibiotic-free swine. *Campylobacter* from antibiotic-free swine had greater prevalence of resistance to clindamycin (13 vs. 4 percent), and environmental samples from antibiotic-free operations had greater prevalence of resistance to azithromycin (34 vs. 15 percent), erythromycin (34 vs. 15 percent), and clindamycin (20 vs. 3 percent). Similar results were observed at slaughter among samples from swine carcasses and the environment. Associations were seen between types of antibiotics used at conventional operations and resistance to the same antimicrobial class isolated from these operations. Antibiotic resistant *Campylobacter* was found among both antibiotic-free and conventionally raised swine during production and slaughter, but there were differences in the antibiotic resistance profiles between the two systems.

Phylogenetic analysis reveals common antimicrobial resistant *Campylobacter coli* population in antimicrobial-free (ABF) and commercial swine systems. M.P. Quintana-Hayashi, S. Thankur. *PLoS One*. 2012, 7(9): 1-6.

Summary: Examines the genetic diversity and persistence of *Campylobacter coli* among swine raised in a conventional setting and those raised in an antibiotic-free setting. Samples were taken from swine on the farm and at slaughter as well as from the environment on the farms and slaughterhouses. About two thirds of *C. coli* isolates were from one distinct group. Antimicrobial resistance was observed among samples from both production systems indicating a shared common ancestry, with some variability in the predominate resistance patterns. Authors conclude that the presence of antimicrobial-resistant *C. coli* in the absence of selective pressure on antibiotic-free operations may be explained by common ancestry of these isolates to those on conventional operations and the persistence of *C. coli* in the farm and slaughter environments.

Isolation and characterization of Methicillin-resistant *Staphylococcus aureus* from pork farms and visiting veterinary students. T.S. Frana, A.R. Beahm, B.M. Hanson, J.M. Kinyon, L.L. Layman, L.A. Karriker, A. Ramirez, T.C. Smith. *PLoS One*. 2013. 8(1): e53738.

Summary: Discusses the prevalence and characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) among pigs and in the environment on pork farms in the Midwestern United States as well as the transmission dynamics of MRSA isolated from these farms. MRSA was detected among pigs and in the environment. Twenty-nine veterinary students visiting the sampled farms provided 604 nasal samples at multiple points before and after visiting pork farms. Among these individuals, there were 27 visits made to MRSA-positive farms and MRSA was detected in the nares of 5 students with at least one visit to a MRSA-positive farm immediately following the visit. MRSA was not detected in the nares of these students more than 24 hours after the visit, potentially indicating short-term contamination of the nasal passage as opposed to colonization. MRSA isolates identified in pigs, the environment, and humans belonged mainly to sequence types (ST) ST398, ST5, ST72 with a high level of concordance between the samples

The drug of choice to treat human infections from Gram-negative bacteria is another third-generation cephalosporin, ceftriaxone. This study examines the association between antibiotic-resistance in *Salmonella* spp. and *Escherichia coli* swine and ceftiofur use in swine operations in North Carolina. Barns were classified based on ceftiofur use rates as rare, moderate, or common with 579, 648, and 672 fecal samples taken from each category respectively. *E. coli* resistant to ceftriaxone was recovered from 45 percent of samples from rare use barns, 73 percent from moderate use barns, and 68 percent from common use barns. *Salmonella* spp. with resistance to ceftriaxone were found in 4.1 percent of samples from rare use barns, 0.15 percent of samples from moderate use barns, and 6 percent of samples from common use barns. Authors suggest that barns with increased ceftiofur use have greater proportions of *E. coli* and *Salmonella* spp. that are resistance to ceftriaxone.

Detection of the staphylococcal multiresistance gene *cfr* in *Proteus vulgaris* of food animal origin. Y. Wang, Y. Wang, C. Wu, S. Schwarz, Z. Shen, W. Zhang, Q. Zhang, and J. Shen. *Journal of Antimicrobial Chemotherapy*. 2011. 66: 2521-2526.

Summary: Details the presence of a plasmid-borne resistance gene commonly found in gram-positive bacteria in the chromosomal DNA of a gram-negative bacterium taken from a pig. The *cfr* gene has the ability to mediate transfer of resistance to linezolid, an antibiotic used in clinical practice to treat human infections caused by gram-positive bacteria resistant to other antibiotics. In this case *cfr* was found in a *Proteus vulgaris* isolate from the nares of a pig raised on a conventional pig operation in China. This finding supports the claim that selective pressure from the use of antibiotics in pig production may allow for the maintenance and transfer of antibiotic resistance among bacteria.

Prevalence and antimicrobial resistance profile of *Campylobacter* spp. isolated from conventional and antimicrobial-free swine production systems from different US regions. D.A. Tadesse, P.B. Bahnson, J.A. Funk, S. Thakur, W.E. Morgan Morrow, T. Wittum, F. DeGraves, P. Rajala-Schultz, and W.A. Gebreyes. *Foodborne Pathogens and Disease*. 2011. 8(3): 367-74.

Summary: Investigates the prevalence and antimicrobial resistance patterns of *Campylobacter* among pigs raised in a conventional environment and in antibiotic-free (ABF) environments at various stages of processing from pre-evisceration to post-chill through the collection of fecal and carcass swab samples. Approximately 95 percent (1034/1087) of isolates available for speciation were found to be *C. coli*, and the remainder was not *C. coli* or *C. jejuni*. 58.9 percent of conventional pigs (252/428) and 53.8 percent (220/411) ABF pigs were positive for *Campylobacter*. Prevalence of *Campylobacter* varied at different stages of production with the chill stage demonstrating the highest prevalence for both types of operations in both regions examined. The most common resistance pattern for *Campylobacter* isolated from ABF operations was resistance to tetracycline only (25.2 percent), erythromycin-tetracycline (10.3 percent), and erythromycin-nalidixic acid-tetracycline (10.3 percent). From conventional operations the dominant resistance patterns were erythromycin-tetracycline (33.4 percent), tetracycline (16 percent) and erythromycin (12.6 percent). Conventional and ABF production systems demonstrated 1.2 and 3.7 percent ciprofloxacin resistance. Of all isolates 2.9 percent (37/1257) were resistant to erythromycin and ciprofloxacin, the drugs of choice for treating human *Campylobacter* infection.

contamination was found between samples, pointing to the need for good hygiene practices at the retail level.

Occurrence and persistence of erythromycin resistance genes (*erm*) and tetracycline resistance genes (*tet*) in waste treatment systems on swine farms. J. Chen, F. C. Michel Jr. S. Sreevatsan, M. Morrison, and Z. Yu. *Microbial Ecology,* 2010.

Summary: This study focuses on how to control antibiotic resistance (AR) that is generated by use of antibiotics in confined animal feeding operations (CAFOs). The authors suggest there are two ways to control AR: reduce the use of antimicrobials on farms or find an effective way to minimize AR dissemination off farms by destroying or containing AR on farms. This study focuses on the latter of those two ways and looks to gain perspective on how well swine farms are containing antibiotic resistance by treating animal manure that is produced in CAFOs before it is being disseminated into the environment. Three swine farms were sampled with different types of waste treatment systems. Upon testing in various stages of waste clean up the authors find that "AR arising from swine-feeding operations can survive typical swine waste treatment processes" and call for treatments that are more functional in destroying AR on farms.

Abundance and diversity of tetracycline resistance genes in soils adjacent to representative swine feedlots in China. N. Wu, M Qiao, B. Zhang, W-D Cheng, and Y-G. Zhu. *Environmental Science and Technology,* 2010.

Summary: Studies the prevalence of tetracycline genes in soil samples from farmlands in the vicinity of nine swine farms located in three cities in China. Finds that 15 tetracycline-resistance genes were commonly detected in soil samples. A strong correlation was found between the concentrations of tetracycline residues, bacterial load and organic matter. Suggests that soils containing bacteria near swine farms may play an important role in the spread of antibiotic resistance and are a large environmental reservoir.

Changes in the use of antimicrobials and the effects on productivity of swine farms in Denmark. F.M. Aarestrup, V.E. Jensen, H-Dorthe Emborg, E. Jacobsen, and H.C. Wegener. *American Journal of Veterinary Research,* 2010. 71:7 726-33.

Summary: Evaluates the changes in antimicrobial use and swine productivity in Denmark between the years 1992 - 2008. In an effort to control the use of antimicrobials in food animal production, Denmark placed a ban on the use of growth promoting antibiotics in January of 2000. In the previous years leading up to the ban, other laws were passed limiting the veterinary profits that could be made on the prescription sale of antibiotics and also included treatment advice for veterinarians to guide the use of antibiotics. The study found there was a greater than 50 percent decrease in the use of antimicrobials per kg of pig produced during the time period from 1992 - 2008 which was associated with the policy to discontinue the use of growth promoting antibiotics. During this time the mortality rate was steady and production increased suggesting that this policy did not have a negative impact on swine production in Denmark.

Ceftiofur use in finishing swine barns and the recovery of fecal *Escherichia coli* or *Salmonella* spp. resistant to ceftriaxone. E.A. Lutz, M.J. McCarty, D.F. Mollenkopf, J.A. Funk, W.A.Gebreyes, and T.E. Wittum. *Foodborne Pathogens and Disease.* 2011. 8(11): 1229-1234.

Summary: Ceftiofur is the only third-generation cephalosporin labeled for veterinary use. It can be used to treat infections from Gram-negative bacteria and is used widely in the swine industry.

sampled was resistant to at least one antibiotic. Prevalence of resistant bacteria was higher among workers or residents of the farms where antibiotics were fed to hogs. Results indicate that farmers have an increased occupational hazard of exposure to antibiotic-resistant bacteria when antibiotics are fed to animals.

Monitoring and source tracking of tetracycline resistance genes in lagoons and groundwater adjacent to swine-production facilities over a 3-year period. S. Koike, I.G. Krapac, H.D. Oliver, A.C. Yannarell, J.C. Chee-Sanford, R.I. Aminov, and R.I. Mackie. *Applied and Environmental Microbiology,* 2007. 73(15): 4813-4823.

Summary: Studies the dissemination of tetracycline-resistance genes from lagoons into the surrounding environment. DNA was extracted and analyzed by real-time quantitative PCR showing a similarity of 99.8 percent for a selected resistance gene between collected groundwater sample DNA and that of the lagoons. States that this is clear evidence that animal waste seeping from lagoons can affect the environment by spreading resistance genes though groundwater contamination.

Antibiotic-resistant *Enterococci* and fecal indicators in surface water and groundwater impacted by a concentrated swine feeding operation. A.R. Sapkota, F.R. Curriero, K.E. Gibson, and K.J. Schwab. *Environmental Health Perspectives*, 2007. 115(7): 104-1045.

Summary: Reviews the risks associated with exposure to manure-contaminated water sources by industrial farms. The authors could not obtain specific data on levels of antibiotics in swine feed because it was premixed and delivered by a contracted integrator, which had deemed antibiotic-usage data proprietary information. Reports that elevated levels of fecal indicators and antibiotic-resistant *Enterococci* were detected in water sources situated down-gradient from a swine facility compared with up-gradient surface water and groundwater. Concludes that "the presence of resistant bacteria in both drinking water and surface water sources contaminated by swine farms could contribute to the spread and persistence of both resistant bacteria and antibiotic resistance determinants in humans and the environment."

Antibiotic resistant bacterial profiles of anaerobic swine-lagoon effluent. J.P. Brooks and M.R. McLaughlin. *Journal of Environmental Quality,* 2009. 38: 2431-2437.

Summary: Focuses on three types of swine farms—farrowing, nursery and finisher. Antibiotic-resistant bacteria were screened for and isolated from all three types of farm lagoons. States that selective pressures appear to have an effect on the amount of resistant isolates recovered from swine-waste lagoons. Nursery lagoons appeared to be most contaminated, with antibiotic-resistant bacteria most likely due to the elevated use of antibiotics in these operations. Finisher farm lagoons contained the lowest concentration, signaling a lower use of antimicrobials in this environment.

Prevalence, numbers and characteristics of *Salmonella* spp. on Irish retail pork. D.M. Prendergast, S.J. Duggan, U. Gonzales-Barron, S. Fanning, F. Butler, M. Cormican, and G. Duffy. *International Journal of Food Microbiology,* 2009. 131: 233-239.

Summary: Explores results of a survey of *Salmonella* in samples of pork from butcher shops and retail markets in Ireland and reports that it was found to contaminate 2.6 percent of samples assayed. *S. Typhimurium* was the dominant serotype found, at a rate of 85 percent; it is also one of the most frequently isolated serotypes from humans in the Irish population. Evidence of cross-

farm sites were resistant to at least one antibiotic, while only one isolate from each of the reference sites showed resistance. Concludes that groundwater on or near swine farms may pose as an environmental pool for antibiotic-resistant *E. coli* and resistance genes.

The effect of subtherapeutic chlortetracycline on antimicrobial resistance in the fecal flora of swine. J.A. Funk, J.T. Lejeune, T.E. Wittum, and P.J. Rajala-Schultz. *Microbial Drug Resistance*, 2006. 12(3): 210-218.

Summary: Studies the occurrence of antimicrobial-resistant *Salmonella* due to the subtherapeutic use of chlortetracycline in the diets of swine. Concludes that "there was a positive association between inclusion of subtherapeutic chlortetracycline in the diet and resistance to multiple antimicrobials."

Isolation of antibiotic-resistant bacteria from the air plume downwind of a swine confined or concentrated animal feeding operation. S.G. Gibbs, C.F. Green, P.M. Tarwater, L.C. Mota, K.D. Mena, and P.V. Scarpino. *Environmental Health Perspectives*, 2006. 114: 1032–1037.

Summary: Studies air samples from upwind, downwind and inside of a confined hog operation. Bacterial samples were tested for antibiotic resistance and *Staphylococcus aureus was* the dominant species recovered. Samples taken within the barn displayed the highest rate of resistance; samples taken up to 150 meters downwind of the barn showed a higher level of resistance than samples taken upwind. Multiple antibiotic-resistant organisms were also found within and around the barn. Concludes that this increase in antimicrobial resistance could have a negative on the health of people who live around these facilities.

Community-acquired MRSA and pig-farming. X.W. Huijsdens, B.J. van Dijke, E. Spalburg, M.G. van Santen-Verheuvel, M.E. Heck, G.N. Pluister, A. Voss, W.J.B. Wannet, and A.J. de Neeling. *Annals of Clinical Microbiology and Antimicrobials*, 2006. 5(26).

Summary: Reports a mother and baby who were found to be carriers of MRSA. A case study followed, finding that the father was a pig farmer, a screening was done to test coworkers, pigs and family members. Three coworkers, eight of 10 pigs and the father were found to be carriers of MRSA. Molecular characterization of the samples clearly revealed transmission of MRSA from pigs to humans. These findings show clonal spread and transmission of MRSA between humans and pigs in the Netherlands.

Are swine workers in the United States at increased risk of infection with zoonotic influenza virus? K.P. Myers, C.W. Olsen, S.F. Setterquist, A.W. Capuano, K.J. Donham, E.L. Thacker, J.A. Merchant and G.C. Gray. *Clinical Infectious Diseases,* 2006. 42: 14-20.

Summary: Studies farmers, meat-processing workers, veterinarians and a control group to determine the extent of exposure to pandemic influenza strains originating from pigs. Finds that farmers are at greatest risk and tend to demonstrate a higher titer to both H1N1 and H1N2 swine influenza virus isolates than control subjects do.

Risk factors for antimicrobial resistance among fecal *Escherichia coli* from residents on forty-three swine farms. T.H. Akwar, C. Poppe, J. Wilson, R.J. Reid-Smith, M. Dyck, J. Waddington, D. Shang, N. Dassie, and S.A. McEwen. *Microbial Drug Resistance*, 2007. 13(1): 69-76.

Summary: Focuses on residents and workers of hog operations that fed antibiotics and those that did not. *E. coli* was obtained from 115 residents and tested for resistance; 25.8 percent of *E. coli*

no effect on average daily gain. The authors note that this study differs from other similar studies, as their noted average daily gains were less than previous reports. Listed explanations include: previous data being biased toward publication of data with positive results; the excellent performance of the control group in the present study; and the fact that current hygienic conditions used exceeded that in previous trials allowing for the control group to perform at a higher level. The authors state that results of the present study indicate that the use of multisite pig production methods greatly reduce pathogen burden on pigs and in turn allows for reduction in use of non-therapeutic antimicrobials.

Productivity and economic effects of antibiotics used for growth promotion in U.S. pork production. G. Y. Miller, K. A. Algozin, P. E. McNamara, and E. J. Bush. *Journal of Agricultural and Applied Economics*, 2003. 35(3): 469-482.

Summary: Studies the use of growth promoting antibiotics (GPA) in pork production. Finds that when GPA are removed from production operations that use less than four different rations (feed) there is a net decrease in return at sale of nine percent. However, when farms use greater than four different rations there is an increase in feed conversion without the use of antibiotics. Furthermore, when farms used greater than four different rations and applied GPA, feed conversion decreased. The authors state "our results imply that antibiotics used for growth promotion are of value mainly when four or fewer different rations are used in finishing."

Antimicrobial resistance in commensal flora of pig farmers. H. Aubrey-Damon, K. Grenet, P. Sall-Ndiaye, D. Che, E. Cordeiro, M.E. Bougnoux, E. Rigaud, Y. Le Strat, V. Lemanissier, L. Armand-Lefèvre, D. Delzescaux, J.C. Desenclos, M. Liénard, and A. Andremont. *Emerging Infectious Diseases,* 2004. 10(5): 873-879.

Summary: Compares the carriage rates of antibiotic-resistant bacteria isolated from pig farmers and non-farmers matched for sex, age and county of residence in France. Finds that farmers carry a higher percentage of resistant commensal bacteria than non-farmers. States that the rate of VRE colonization did not differ between farmers and non-farmers and that this finding suggests that the 1997 ban of avoparcin was effective.

Airborne multidrug-resistant bacteria isolated from a concentrated swine feeding operation. A. Chapin, A. Rule, K. Gibson, T. Buckley, and K. Schwab. *Environmental Health Perspectives*, 2005. 113: 137–142.

Summary: Reports the results of studies air samples taken within confined hog operations for antibiotic-resistant bacteria. Ninety-eight percent of bacteria sampled had resistance to at least two antibiotics used in animal production and a greater potential for worker exposure to resistant bacteria, suggesting that exposure to air from swine operations may allow multidrug-resistant bacteria to be transferred from animals to humans. Notes that "these data are especially relevant to the health of swine CAFO [concentrated animal feeding operations] workers, their direct contacts in the community, and possibly nearby neighbors of swine CAFOs."

Detection and occurrence of antimicrobially resistant *E. coli* in groundwater on or near swine farms in eastern North Carolina. M.E. Anderson and M.D. Sobsey. *Water Science and Technology*, 2006. 54(3): 211-218.

Summary: Compares the extent of groundwater contamination from antibiotic-resistant *E. coli* from industrial swine farms and reference sites. Sixty-eight percent of the *E. coli* from the swine

SWINE

Ways in which swine production affects air, water and farm workers.

An outbreak of multidrug-resistant, quinolone-resistant *Salmonella enterica* serotype typhimurium DT104. K. Molbak, D.L. Baggesen, F.M. Aarestrup, J.M. Ebbesen, J. Engberg, K. Frydendahl, P. Gerner-Smidt, A.M. Petersen, and H.C. Wegener. *New England Journal of Medicine,* 1999. 341: 1420-1425.

Summary: Reviews a 1998 *Salmonella enterica* serotype typhimurium DT104 outbreak in Denmark. The outbreak had 25 confirmed cases, with 11 patients hospitalized and two deaths. Previous cases were resistant to five antibiotics; however, cases in this outbreak also were resistant to nalidixic acid and had reduced susceptibility to fluoroquinolones. Analysis traced the infection to a swine herd delivered to a slaughterhouse and the resulting retail pork was found to be the common food source.

Concentrated swine-feeding operations and public health: A review of occupational and community health effects. D. Cole, L. Todd, and S. Wing. *Environmental Health Perspectives,* 2000. 108: 685-699.

Summary: Reviews the effects of industrial farms on community health. States that there are many potential routes of community exposure to industrial farming hazards and that people residing near swine farms may be exposed to these agents through pathways such as airborne contaminants produced by building ventilation fans, soil transport of microbes from land-applied wastes and leaking lagoons that contaminate groundwater. States that more research is needed to determine the far-reaching effects of industrial farms on community health.

Occurrence and diversity of tetracycline-resistance genes in lagoons and groundwater underlying two swine production facilities. J.C. Chee-Sanford, R.I. Aminov, I.J. Krapac, N. Garrigues-Jeanjean, and R.I. Mackie. *Applied and Environmental Microbiology*, 2001. 67(4): 1494-1502.

Summary: States that 25 percent to 75 percent of antimicrobials administered to food animals are poorly absorbed in the gut and are excreted in feces. These unaltered substances are then applied to land by spreading of manure. Finds that a broad range of tetracycline-resistance genes occurred in two swine-waste lagoons and that upon release into the environment these genes can potentially mobilize and persist. Data suggest that the presence of the resistance genes is due to seepage and movement of groundwater underlying the lagoons and that it may be substantial, as resistance genes were found in a well 250 meters downstream of the lagoon sampled.

Effects of administration of antimicrobials in feed on growth rate and feed efficiency of pigs in multisite production systems. S.S. Dritz, M.D. Tokach, R.D. Goodband, and J.L. Nelssen. JAVMA 2002. 220:11 1690-95.

Summary: A study consisting of 10 trials involving a total of 24,099 finishing and nursery pigs. Trials involving pigs feed antimicrobials were selected based on commonly used production system regimens. A control group was also included that was not administered antibiotics unless necessary due to disease and then at a therapeutic dose. When all data were compiled, only nursery pigs showed an increase in average daily gain when given antibiotics in feed. Feed efficiency was lower in all nursery groups given antibiotics in feed compared to the control and feed efficiency was not significantly different in either finishing or nursery groups between control and treated animals. It was concluded that giving antibiotics in feed to finishing pigs had

include: 1) banning the use of antimicrobials as growth promoters in food-animal production; 2) require veterinarian prescription and oversight for administration of antibiotics to food-animals; and, 3) drastically limit the use of antibiotics that critically important for human health.

antimicrobial resistance genes was also reported at varying levels. Researchers demonstrated transfer of the gene that confers resistance to tetracycline, between CoNS and a strain of *Enterococcus*. CoNS may function as a reservoir of antimicrobial resistance genes.

Correlation between upstream human activities and riverine antibiotic resistance genes. A. Pruden, M. Arabi, H.N. Storteboom. *Environmental Science and Technology*. 2012. E-publication.

Summary: Sediment samples collected at a range of sites encompassing pristine areas and those tainted by human activity within the South Platte River Basin were analyzed for the presence of antibiotic resistance genes (ARGs) to examine correlations between ARGS and upstream sources of ARGs in a watershed. Researchers examined the presence mobile genetic elements conferring resistance to sulfonamide and tetracycline antimicrobials. Information about the proximity of sampling sites to animal feeding operations, wastewater treatment plants, and fish hatchery and rearing units was determined for each sampling site. Increased presence of the gene for sulfonamide resistance was associated with upstream animal feeding operations and wastewater treatment plants. Prevalence of the tetracycline resistant gene was not associated with upstream sites. The findings indicate the contribution of animal feeding operations and wastewater treatment plants to the dissemination of ARGs into the environment through water ways. These findings suggest the importance of water to transport ARGs which may contribute to the disease burden of antibiotic-resistant infections in humans.

Urine from treated cattle drives selection for cephalosporin resistant Escherichia coli in soil. M. Subbiah, D.H. Shah, T.E. Besser, J.L. Ullman, D.R. Call. PLoS One. 2012. 7(11): e48919.

Summary: The study looked at giving the results of giving cattle ceftiofur, a commonly used cephalosporin antibiotic. They monitored the drug and its breakdown products in urine. Ceftiofur-resistant E. coli from the animals was found in the environment and the drug could remain in the soil for days to weeks, depending on the temperature. To demonstrate transmission, bedding was sprayed with a suspension of ceftiofur-resistant E. coli and allowed to dry. Then calves were introduced to the environment and by the second day, the calves were also shedding ceftiofur-resistant E. coli in their feces. These results show that when ceftiofur is administered to animals its breakdown products are excreted through urine and may persist in the soil or environment for a prolonged period allowing for selection of ceftiofur-resistant bacteria and exposure of the animals to these bacteria through their environment.

Extended-spectrum cephalosporin-resistant gram-negative organisms in livestock: An emerging problem for human health? S.N. Seiffert , M. Hilty, V. Perreten, A. Endimiani. 2013. *Drug Resistance Updates*. Epub.

Summary: There is an increase in the prevalence of gram-negative organisms that produce extended-spectrum-β-lactamases (ESBL), which confers resistance to extended-spectrum cephalosporins (main antibiotics to treat infections caused by gram-negative bacteria). The contribution of use of antibiotics in food-animal production as a major contributing factor to the increase in extended-spectrum cephalosporin-resistant gram-negative bacteria found among food animals and humans is modeled. Detailed information regarding the prevalence of resistant *E. coli*, *Salmonella*, and *Acinetobacter* among food-animals and humans as well as antibiotic use is presented by country. A summary of the evidence linking resistant bacteria found among food animals to that found in humans is provided and transmission routes between food-animals and humans are reviewed. Strategies for controlling the increase and spread of resistant bacteria

antibiotic resistance or the presence of resistance genes among the three types of chickens sampled were found. *Campylobacter* isolated from pigs demonstrated resistance to ampicillin, ciprofloxacin, clindamycin, erythromycin, streptomycin, and tetracycline. Although differences in resistance and resistance genes were present, authors report that isolates from poultry and pigs were of related clonal groups. Differences may be due to differences antimicrobial use among these animal types.

The shared antibiotic resistome of soil bacteria and human pathogens. K.J. Forsberg, A. Reyes, B. Wang, E.M. Selleck, M.O.A. Sommer, G. Dantas. *Science*. 2012, 337: 1107-1111.

Summary: Antibiotic-resistance genes identified among non-disease causing bacteria commonly found in soil display were identical to the antibiotic-resistance genes among human pathogens. The genes represent a variety of known mechanisms of resistance. The findings support evidence indicating the potential for exchange of antibiotic-resistance genes between non-pathogenic environmental bacteria, such as those in soil, and human pathogens, either directly or indirectly. This indication of the potential for exchange raises concerns about environmental use of antibiotics, including within livestock production, which may consequently contribute to selective pressure and increased presence of antibacterial resistance among harmless and disease causing bacteria.

Livestock density as risk factor for livestock-associated methicillin-resistant *Staphylococcus aureus*, the Netherlands. B.J. Feingold, E.K. Silbergeld, F.C. Curriero, B.A.G.L. van Cleef, M.E.O.C. Heck, J.A.J.W. Kluytmans. *Emerging Infectious Diseases*. 2012. E-pub.

Summary: Reports results from a study conducted to affirm previously reported individual-level risk factors and identify other factors for livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) as compared to other types of MRSA. The study examines 27 cases of LA-MRSA carriage (clonal complex 398) and 60 cases of non-LA-MRSA in the Netherlands. Based on spatial and statistical analyses, contact with pigs, contact with cows, and living in a rural area were important individual-level factors associated with increased odds of LA-MRSA. Additionally, increasing density of pigs, cows, or veal calves in the municipality of residence was also associated with increased odds of LA-MRSA. These results carry important policy implications for the Netherlands and other areas with high densities of livestock including the United States, but should be interpreted with caution as they are based on small numbers.

Multidrug-resistant coagulase-negative Staphylococci in food animals. K. Bhargava, Y. Zhang. *Journal of Applied Microbiology*. 2012. 113(5): 1027-1036.

Summary: Although coagulase-negative Staphylococci (CoNS) are not the most pathogenic species of staphylococci, CoNS are common commensal bacteria carried by animals and often carry genes conferring antimicrobial-resistance that are easily transferable to other, more pathogenic bacteria. Among 87 CoNS isolates resistant to oxacillin and obtained from a range of food animals, 79 percent were resistant to ampicillin, 92 percent to penicillin, 68 percent to tetracycline, 37 percent to erythromycin, 28 percent to clindamycin, and 15 percent to quinupristin-dalfopristin. Approximately 54 percent of isolates were resistant to at least three antimicrobial classes and were labeled multidrug resistant (MDR). MDR isolates were found among most animal types with 80 percent of isolates from chicken and turkey classified as MDR as well as 67 percent from duck, 62 percent from goats, 60 percent from sheep, 57 percent from pigs, and 37 percent from cattle. Prevalence information regarding the presence of specific

streptomycin (34 percent), and ampicillin (24 percent) most common. Of isolates from humans, 65 percent were not resistant to any of the 15 antimicrobials tested as compared to 20 percent of animal isolates. However, the proportion of pan-susceptible isolates decreased over time from 74 percent in 1950-1959 to 19 percent in 2000-2002, while the proportion of multidrug-resistant isolates increased during the same period (7 to 64 percent). When resistance trends were examined over time, animal isolates demonstrated an increasing trend for resistance to 11/15 antimicrobial drugs. Isolates from humans showed an increasing trend for resistance to three of the same drug classes (ampicillin, streptomycin, and tetracyclines) for which animal isolates showed an increasing trend, but no additional classes of drugs. Authors conclude these findings provide information to support the development of resistance over time and that antimicrobial resistance in *E. coli* can be temporally linked with use of antimicrobials.

Selective pressure of antibiotic pollution on bacteria of importance to public health. A. Tello, B. Austin, T.C. Telfer. *Environmental Health Perspectives*. 2012, April 16 Epub.

Summary: Antibiotics used in agriculture are commonly released into the environment where many bacteria can survive and grow. The exposure of these bacteria to antibiotics at high enough concentrations is thought to produce a selective pressure that would select for antibiotic-resistant bacteria and inhibit growth of wild-type bacteria that is not resistant to the antibiotic. This study gathered information regarding concentrations of ciprofloxacin, erythromycin, and tetracycline in different environments from literature and examined the selective pressure that may be present on clinically important bacteria found in the respective environments at the concentrations identified and at concentrations considered to be action limits in environmental risk assessment. Antibiotics at the concentrations measured in river sediments are estimated to inhibit growth of wild-type bacteria in up to 60 percent of bacterial genera. High proportions of bacterial genera were also found to be inhibited at concentrations of antibiotics found in swine feces lagoons (92 percent), liquid manure (100 percent) and farmed soil (30 percent). When authors compared measured concentrations of ciprofloxacin and tetracycline in environments with levels thought to inhibit 100 percent growth of wild-type populations among bacterial genera, wild-type populations of several species would be completely inhibited. At concentrations of ciprofloxacin and erythromycin used as soil action limits in environmental risk assessment, wild-type populations were inhibited for 76 and 25 percent of bacterial genera. Authors conclude the concentrations of antibiotics may be sufficient to apply a selective pressure to bacterial populations found in the same environments, many of which are clinically important. Further, environments including river sediments, liquid manure, and farmed soil may be important areas of concern based on reported concentrations of antibiotics. Moving forward, antibiotic resistance should be considered as part of environmental risk assessments and efforts to reduce antibiotics in the environment should be strongly considered to curb the increase of antibiotic resistance.

Antimicrobial susceptibilities and resistant genes in *Campylobacter* strains isolated from poultry and pigs in Australia. A. Serwaah Obeng, H. Rickard, M. Sexton, Y. Pang, H. Peng, M. Barton. *Journal of Applied Microbiology*, 2012. 78(8):2698-705. [June 2 E-pub ahead of print]

Summary: Describes the presence of and antibiotic resistance profiles of *Campylobacter* species isolated from free range meat chickens, free range egg layers, and commercial meat chickens in Australia. Results concerning antimicrobial resistance genes found among *Campylobacter* isolated from pigs from the same area are also presented. Resistance to lincomycin, ampicillin, and tetracycline was observed in all three types of chickens. No differences in the presence of

Feather meal: A previously unrecognized route for reentry into the food supply of multiple pharmaceuticals and personal care products (PPCPs). D.C. Love, R.U. Halden, M.F. Davis, K.E. Nachman. *Environmental Science & Technology*, 2012. 46: 3795-3802.

Summary: Feather meal is created by rendering poultry feathers and included as an animal feed ingredient in addition to other uses. Authors examined commercially available feather meal products for the presence of pharmaceuticals and personal care products (PPCP). Researchers found that residues of 17 of 46 antimicrobials, representing 6 different drug classes, were detectable in 100% (12/12) of commercial feather meal samples tested. Most of the antimicrobials detected in these samples are from drug classes approved for use in industrial poultry production. PPCP were identified in 83 percent (10/12) of samples. PPCP identified included an antidepressant, an antihistamine, fungicide, analgesic, sex hormone, and stimulant. In order to show the importance of the presence of antimicrobials in commercial feather meal products, researchers performed other experiments that showed that when a susceptible strain of *Escherichia coli* was exposed to autoclaved feather meal, a process that approximates rendering, growth of the bacteria was inhibited. When a resistant strain of *E. coli* was grown with feather meal, growth of the bacteria was not inhibited. The results presented in this study indicate the presence of active antimicrobials and other products in commercial feather meal after rendering.

A review of antibiotic use in food animals: Perspective, policy, and potential. T.F. Landers, B. Cohen, T.E. Wittum, and E.L. Larson. *Public Health Reports*. 2012. 127(1): 4-22.

Summary: Reviews the published information on the use of antibiotics in food animals as well as policies related to their use, and summarizes the potential impact on human health. Although there is widespread use of antibiotics, it appears that a lack of reliable information to indicate the quantity and patterns of antibiotic use in food animals is available. Estimates for the proportion of antibiotics used in food animals range from 17.8 million to 31.9 million pounds annually with varying proportions estimated to be used sub-therapeutically. Although benefits of antibiotic use are often put forward and deserve consideration, most of the claims have not been substantiated and a body of literature exists that lends support to an association between antibiotic use in food animals and antibiotic resistance bacteria in humans. As few benefits have been realized and a large concern based on scientific research has grown, a large body of policies and recommendations has been put forward and are presented in detail. Based on the literature available, authors 1) recommend that the scientific community develop a plan to generate the scientific data that is missing to date; 2) urge the U.S. government and other funding agencies to place more of an emphasis on funding scientific work to address the use of antibiotics in food animal production; and, 3) confront and deal with obstacles to collecting data and conducting scientific research. The review concludes that it is imperative that the use of antibiotics in food animals be recognized as an important contributor to antibiotic resistant infections in humans and be addressed directly.

Antimicrobial drug resistance in *Escherichia coli* from humans and food animals, United States, 1950-2002. D.A. Tadesse, S. Zhao, E. Tong, S. Ayers, A. Singh, M.J. Bartholomew, P.F. McDermott. *Emerging Infectious Diseases*. 2012, 18(5): 741-749.

Summary: Explores and describes the emergence of antimicrobial resistance among 1,729 *Escherichia coli* isolates obtained from animals and humans (983) between 1950 and 2002. Overall, 934/1729 (54 percent) of animal isolates were resistant to at least one antimicrobial drug with resistance to older drugs including tetracycline (41 percent), sulfonamide (36 percent),

Food animals and antimicrobials: Impacts on human health. B.M. Marshall and S.B. Levy. *Clinical Microbiology Reviews*. 2011, 24(4): 718-733.

Summary: Reviews literature on the link between nontherapeutic antimicrobial (NTA) use in food-animal production, including aquaculture, and the emergence of antibiotic-resistant bacteria in humans and concludes that a wealth of evidence exists to support this link. Use of antibiotics is a powerful force in the selection of resistant bacteria and use anywhere can lead to resistance at point of use and in other areas. Identifies gaps in knowledge related to NTA use highlighting the lack of research regarding genetic infrastructure and spread between commensal and environmental bacteria.

Antibiotic resistance, gene transfer, and water quality patterns observed in waterways near CAFO farms and wastewater treatment facilities. B.M. West, P. Liggit, D.L. Clemans, and S.N.Francoeur. *Water, Air & Soil Pollution*. 2011. 217(1-4): 473-489.

Summary: Reports findings from a water quality assessment study that sampled up and downstream of waste water treatment plants (WWTP) and locations affected by confined animal feeding operations (CAFO) and reference locations unaffected by CAFOs. Chemical and biological water quality indicators were evaluated. Sites up and downstream of WWTPs met current chemical and biological water quality standards and were considered environmentally healthy. Fecal coliform density ranged from 70-2,300 CFU/100 ml in these locations. High, but similar, levels of drug-resistant fecal coliforms were found up and downstream of WWTPs. In contrast, sites near CAFOs had reduced water quality compliance and fecal coliform levels ranged from 700 CFU/100 ml to too numerous to count. CAFO affected sites had much higher levels of multi-drug resistant bacteria (42 percent) as compared with agricultural sampling sites not affected by CAFOs (17 percent). In laboratory experiment, antibiotic-resistant bacteria collected at all locations demonstrated the ability to transfer resistance genes. Concludes that surface waters may be an important source of human exposure to antibiotic-resistant bacteria and monitoring of antibiotic-resistance should become part of the standard monitoring of waterways.

In-feed antibiotic effects on the swine intestinal microbiome. T. Looft, T.A. Johnson. H.K. Allen, D.O. Bayles, D.P. Alt, R.D. Stedtfeld, W.J. Sul, T.M. Stedtfeld, B. Chai, J.R. Cole, S.A. Hashsham, J.M. Tiedje, T.B. Stanton. *Proceedings of the National Academy of Sciences*. 2012. 109(5): 1691-1696.

Summary: Supplementation of livestock feed with antibiotics may lead to changes in the commensal bacteria present in the gastrointestinal tract of animals receiving such supplemented feed, which in turn could lead to increases in antibiotic resistance genes and transfer to pathogens. This study examined the gut microbiota of piglets given feed supplemented with ASP250 (a mix containing chlortetracycline, sulfamethazine, and penicillin) and a group of piglets given the same feed without ASP250. A fecal sample was taken before piglets received any ASP250 and three more times over a 21days. By day 14, the microbial make up found in samples from the two groups of pigs were found to be different. A greater abundance of 6 resistance-gene types were found at day 14 in the medicated animals as compared to the non-medicated animals even though no difference in these gene types was seen at day 0. Authors conclude that dosing with antibiotic supplemented feed increases the abundance and diversity of antibiotic resistance genes, and promotes changes in the make-up of the microbiome.

Effects of restricted antimicrobial exposure on antimicrobial resistance in fecal Escherichia coli from feedlot cattle. P.S. Morley, D.A. Dargatz, D.R. Hyatt, G.A. Dewell, J.G. Patterson, B.A. Burgess and T.E. Wittum. *Foodborne Pathogens and Disease,* 2011. 8-1: 1-12.

Summary: A study on two types of feedlot cattle. Conventional was raised and fed a diet with antibiotics while natural was raised and fed a diet of no antibiotics and were only exposed to antibiotics if disease occurred. The authors conclude that there was no difference in resistance to collected *E. coli* from either group in the study and suggest that conventional feedlot production methods do not predictably increase the prevalence of antimicrobial resistance in *E. coli* when compared to animals raised with restricted exposure to antibiotics.

The study design assigned pens to the natural cattle that may have previously housed cattle fed a conventional diet and no documentation is made on if pens were cleaned before study groups were placed. Cattle from the conventional group were also sometimes clustered in pens adjacent to the natural group to facilitate feeding. Also, after antibiotics were administered to sick cattle they were returned to their previous pens, which may have exposed other cattle in the group to the treated animal's microbial flora. While antibiotic susceptibility testing showed little to no difference (higher resistance to tetracycline and chloramphenicol was observed in conventional cattle) in resistance patterns of collected *E. coli*, the conventional cattle were fed Tylosin, a macrolide antibiotic and there were no macrolides in the susceptibility panel of testing. The authors do not address if the naturally raised cattle could have been exposed to bacteria from previous cattle fed antibiotics that were housed in the same pens.

Also of note is that this study was designed around "natural" raised and "conventional" raised cattle. These definitions are synonymous with the only differences being that "natural" products are minimally processed and do not contain artificial or synthetic ingredients or coloring additives; however, in this study they did attempt to keep the "natural" beef antibiotic free.

Dose imprecision and resistance: Free-choice medicated feeds in industrial food animal production in the United States. D.C. Love, M.F. Davis, A. Bassett, A. Gunther, and K.E. Nachman. *Environmental Health Perspectives*, 2011. 119(3):279-283.

Summary: Food animals in the US are often provided food that includes antibiotics and antiparasitic drugs on a "free choice" basis meaning the animals decide when to eat food and how much of it to consume. This practice is referred to as using free-choice medicated feeds (FCMF) and is shown to result in imprecision of drug intake leading to under and over-medication of animals. Imprecision in dosing of animals can lead to the development of antibiotic-resistant microorganisms or the presence of drug residues in food products. Various factors including labeling, veterinary oversight, feed characteristics, animal and herd behavior, behavior of workers, and drug pharmacokinetics may affect the dose ingested and received by the animal. Little oversight or control of FCMF exists in the US and no federal requirements exist for reporting use of antimicrobial drugs in animal production. This is despite the proposal of the Preservation of Antibiotics for Medical Treatment Act (PAMTA) introduced to Congress in 2009 and a statement from the US Food and Drug Administration (FDA) that "the overall weight of evidence available to date supports the conclusion that using medically important antimicrobial drugs for production purposes is not in the interest of protecting and promoting the public health." The authors conclude that use of FCMF poses an unnecessary risk to public health and that a more appropriate system of medicating animals should be used only when necessary to treat clinically diagnosed disease.

Antibiotic resistance, gene transfer, and water quality patterns observed in waterways near CAFO farms and wastewater treatment facilities. B.M. West, P. Liggit, D.L. Clemans, and S.N. Francoeur. *Water, Air, and Soil Pollution.* 2010.

Summary: In this study, water quality indicators were measured in locations impacted by confined animal feeding operations (CAFOs), nearby reference sites and sites located upstream and downstream of two wastewater treatment facilities. Selected study sites that were impacted by agriculture including three waterways showed levels of phosphorus higher than all other sites, and fecal coliform densities ranged from 70 to >7,900 CFU/100ml. In instances of rain events, densities of fecal coliforms from samples ranged from 700 to "too numerous to count." Samples containing 70 to 2,300 CFU/100ml were taken from waterways near wastewater treatment plants. Agriculturally impacted sites had a significantly greater proportion of isolates that showed resistance to multiple antibiotics. In assays designed to screen for the transferability of DNA encoding resistance, environmental isolates were found to be very likely to contain elements allowing for this exchange. The authors suggest that testing for antibiotic resistance genes in bacterial strains become part of standard methods when examining water quality in areas at risk for pollution from human and/or animal waste.

CTX-M-type extended-spectrum ß-lactamases present in *Escherichia coli* from the feces of cattle in Ohio, United States. T.E. Wittum, D.F. Mollenkopf, J.B. Daniels, A.E. Parkinson, J.L. Mathews, P.R. Fry, M.J. Abley and W.A. Gebreyes. *Foodborne Pathogens and Disease.* 2010.

Summary: CTX-M extended-spectrum ß-lactamases are enzymes produced by bacteria that allow them to inhibit the antimicrobial effects of penicillins and cephalosporin drugs. This is of public health concern as expanded-spectrum cephalosporins are the treatment of choice for infections such as salmonellosis in children. In this study, samples from bovine fecal samples were screened for CTX-M producing strains of *E. coli*. Results show that 6 percent (3/50) of fecal samples collected harbored CTX-M genes. It has been hypothesized that ceftiofur use in livestock populations may provide the necessary selection pressure for such resistance genes as CTX-M to disseminate. Results from this study support the hypothesis as *E. coli* harboring CTX-M was isolated from a calf that was recently treated with ceftiofur therapy.

Producer attitudes and practices related to antimicrobial use in beef cattle in Tennessee. A.L. Green, L.R. Carpenter, DE. Edmisson, C.D. Lane, M.G. Welborn, F.M. Hopkins, D.A. Bemis and J.R. Dunn. *JAVMA.* 237 (11): 1292-1298.

Summary: This study involved a sampling by mail of 3,000 beef producers across the state of Tennessee, with 1,042 returned. The results showed 56.3 percent of beef cattle operations reported having used antimicrobials within the past year. Producers with multiple operation types (MOT) were more likely than producers with only cow-calf operations to have administered antibiotics either by mouth or by injection within the past year. The MOT producers were also slightly more likely to agree that antibiotics are not working as effectively than in the past. Herd size also had a positive correlation with antimicrobial use. The authors suggest that beef quality assurance programs should be employed and an effort to reach producers not involved in these programs should be explored. Additionally, efforts to reduce antibiotic use among producers may be found through educational efforts focused on practical, cost and labor effective alternatives such as a focus on bio-security, vaccination and low-stress handling of livestock and decreased stocking density to minimize disease transmission.

meters from lagoons. The authors conclude that these occurrences of antibiotics at the farm surface need closer study as they may affect the ecosystem and microbial community including the development of antibiotic resistance.

The interface between veterinary and human antibiotic use. T.R. Shryock and A. Richwine. *Annals of the New York Academy of Sciences,* 2010. 1-14.

Summary: This review looks at the overlaps between human and veterinary medicine and how current views are redirecting company pipelines in regards to new veterinary antimicrobial drug discovery. The authors state that the listing of "critically important" antibiotics by the World Health Organization has led to a prejudice against the food animal use of specific antimicrobial classes. The authors state several possible remedies to use in parallel or in replacement of these antibiotic classes including novel antibiotic development, vaccines, immunomodulators, bacteriophages and probiotics. A resounding theme is the ever-increasing demand of animal-derived protein and that the current and future availability of novel antimicrobial agents for use in food animal production be considered from this perspective. Other key points addressed include appropriate risk assessment before regulation, as uninformed decisions may discourage innovation of new antibiotics and that industry should apply the use of risk management interventions such as appropriate antibiotic use guidelines.

The effects of moral obligations to others and others' influence on veterinarians' attitudes toward and recommendations to utilize antibiotics in feedlot cattle. J-S. Jan, Wm. A. Mcintosh, H. M. Scott, and W. Dean. *Journal of Rural Social Sciences*. 2010. 25 (2): 122-148.

Summary: A questionnaire was sent to feedlot veterinarians querying about areas of social pressure that may affect behavior in treatment of animals. Outcomes suggest that pressure from pharmaceutical companies led to a less likely scenario that veterinarians would have a positive attitude toward using antibiotics in acutely sick cattle. The opposite was seen when rule and norm-making organizations such as veterinary professional organizations, the FDA or state licensing boards applied pressure in that veterinarians' attitudes were positively affected toward the use of antibiotics. Favorability toward using antibiotics was also seen when pressure from feedlot managers and retained owners of cattle was applied but only when moral obligations to these clients were taken into consideration. Otherwise these pressures had little influence on attitudes or recommendations.

Tetracycline and sulfonamide antibiotic resistance genes in livestock lagoons of various operation type, configuration, and antibiotic occurrence. C.W. McKinney, K.A. Loftin, M.T. Meyer, J.G. Davis, and A. Pruden. *Environmental Science & Technology*. 2010. 44: 6102-6109.

Summary: The purpose of this study was to look at waste lagoons among various livestock facilities and examine the behavior of *tet* (tetracyclines) and *sul* (sulfonamide) antibiotic resistance genes (ARGs) over the course of one year. ARG concentrations were significantly higher in lagoon samples from conventional dairy farms compared to organic. Chicken layer operation lagoons had the lowest detectable levels of *tet* and *sul* ARGs, while the highest were in swine lagoons. In general *sul* ARGs were more recalcitrant than *tet* ARGs. The study demonstrated that liquid manure lagoons may show some promise in reducing *tet* ARGs as passing waste though several lagoons decreased *tet* ARGs; however, when compared to sediment samples taken upstream from the facilities, the lagoon water samples still contained three to five times higher *tet* ARGs.

Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. J.C. Chee-Sanford, R.I. Mackie, S. Koike, I.G. Krapac, Y.F. Lin, A.C. Yannarell, S. Maxwell and R.I. Aminov. *Journal of Environmental Quality,* 2009. 38: 1086-1108.

Summary: This review article can be broken down into three parts:

1) Dissemination of antimicrobial residues into the environment: Antibiotics fed to food animals are not always fully absorbed and will be excreted in waste. This waste is often applied to the environment as a disposal and fertilization technique. During this process excreted antibiotics that have not broken down during storage of waste are applied to the environment. Numerous studies are cited where antibiotic residues were found in soil and water near or on concentrated animal feeding operations (CAFO).

2) Resistance characteristics and presence of bacteria in CAFO and their transport into the environment: Antibiotic resistance in animals is likely to arise among commensal bacteria as there is a large pool in the gut (often $>10^{14}$) and antibiotic resistance may be selected for each time an antibiotic is administered regardless of the animals health. This is the most likely pathway for the development of pathogen resistance as commensal bacteria may transfer mechanisms of resistance to bacterial pathogens. It is well documented that these bacteria may survive waste treatment methods and are applied to the soil that may have harmful environmental implications.

3) Antibiotic resistance gene transfer in the environment: By applying animal waste to the environment a pool is created that holds a potentially significant amount of resistance genes; however, the transfer of these mechanisms into commensal bacteria of the environment is relatively unknown. Studies are listed showing that the transfer of resistance genes does occur between bacteria of different genera in such areas as soil and groundwater. The authors conclude that although the impacts from antibiotic use in food animal production and the effects on the environment are not completely clear, there are established studies pointing to an increase of incidences in antibiotic resistance.

Sublethal antibiotic treatment leads to multidrug resistance via radical-induced mutagenesis. M.A. Kohanski, M. A. DePristo and J.J. Collins. *Molecular Cell*, 2010. 37:311-320

Summary: Looks at mutation rates of *E. coli* exposed to sublethal doses of different antibiotics. Finds that when sublethal doses of antibiotics were given, cell production of radical oxygen species (ROS) occurred, leading to mutations. ROS can damage DNA, causing a mutation in such a way that the cells may acquire resistance to classes of antibiotics different from those with which they are being treated. Gives a clinical example of incomplete treatment with antibiotics (e.g., a missed pill), but one could postulate that in food animal production, where subtherapeutic levels of antibiotics are given for the purpose of growth promotion, this event may also occur.

Use and environmental occurrence of antibiotics in freestall dairy farms with manured forage fields. N. Watanabe, B.A. Bergamaschi, K.A. Loftin, M.T. Meyer, and T. Harter. *Environmental Science and Technology,* 2010. 44:17 6591-6600.

Summary: Investigates the use and occurrence of antibiotics in concentrated dairy feeding operations to assess their potential transport into first-encountered groundwater. The study finds that wide arrays of antibiotics are used in the farms leading to several hundred grams of antibiotics being excreted per farm per day. Samples containing sulfonamides, tetracyclines and lincomycin were most frequent; however, it appeared that the occurrence of antibiotics in collected samples was limited to farm boundaries and were usually associated with lagoons, hospital pens and calf hutches. There was detection of antibiotics in shallow groundwater 10

studies a decline in resistance has been shown when antibiotics (selective pressures) were removed from diets of animals, but this may sometimes take years to see a marked decrease. In summary feeding of certain diets and addition of certain sub-therapeutic levels of antibiotics in feed will increase the rate of resistance in *E. coli*.

The effects of transport and lairage on counts of *Escherichia coli* O157 in the feces and on the hides of individual cattle. N. Fegan, G. Higgs, L. Duffy and R.S. Barlow. *Foodborne Pathogens and Disease,* 2009. 6(9):1113-1120.

Summary: Reports on a study in which *E. coli* O157 rates from feces and from hides of cattle were monitored to determine whether a change occurred during transport from the feedlot to slaughter. Concludes that "transport and lairage did not lead to an increase in the number or isolation rate of *E. coli* O157 from cattle."

Comparison of the prevalence of bacterial enteropathogens, potentially zoonotic bacteria and bacterial resistance to antimicrobials in organic and conventional poultry, swine and beef production: a systematic review and meta-analysis. I. Young, A. Rajic, B.J. Wilhelm, L. Waddell, S. Parker, and S.A. McEwen. *Epidemiol. Infect.,* 2009. 137: 1217-1232.

Summary: A systematic review of the literature in comparing organic and conventional meats. Finds that the prevalence of *Campylobacter* was higher in organic broiler chickens at slaughter, but a difference was not seen in retail chicken. *Campylobacter* from conventional retail chicken was more likely to be ciprofloxacin resistant. Furthermore, bacteria isolated from conventional food animal production were found to exhibit higher levels of antibiotic resistance. The authors conclude that further research is necessary in this area as this type of data from other food-animal species was limited or inconsistent.

The transformation of U.S. livestock agriculture: Scale, efficiency, and risks. J.M. MacDonald and W.D. McBride. *Economic Information Bulletin Number 43,* United States Department of Agriculture, 2009.

Summary: Report from the United States Department of Agriculture detailing the nature, causes and effects of structural changes in livestock production. States that due to the increase seen in farm size, livestock wastes are becoming geographically concentrated in the US and the application of these wastes to land poses risks to air and water resources. Large-scale operations are more likely to see a rapid spread of disease due to the concentration of animals and tend to administer sub therapeutic doses of antibiotics routinely in feed and water to animals to promote health and prevent disease. These antibiotics may enter the environment though manure application and overuse may contribute to increased resistance in animal and human pathogens. Other technologies, including better sanitation and testing procedures, can be substituted for these practices in some production stages especially in poultry production. These practices, used in most operations not providing their animals sub therapeutic antibiotics, include: the testing of feed for specific pathogens; testing of flocks routinely for disease; cleaning out and sanitizing houses after each flock; and typically were required to have a hazard analysis and critical control point plan in place to direct food safety measures. The farms that do not rely on sub therapeutic antibiotics for disease prevention were nearly twice as likely to follow these procedures as those farms that used sub therapeutic antibiotics.

are presented as bacteria of concern related to food animal production and human health. Use of antibiotics in the beef cattle, dairy cattle, swine, and poultry industry is discussed. Challenges regulators, the animal production industry, and consumers to take steps in limiting risks based on science-based information.

Associations between antimicrobial resistance genes in fecal generic *Escherichia coli* isolates from cow-calf herds in western Canada. S.P. Gow, C.L. Waldner, J. Harel and P. Boerlin. *Applied and Environmental Microbiology,* 2008. 74(12): 3658-3666.

Summary: Studies antimicrobial-resistance gene distribution among cow-calf herds in western Canada. Finds that 65 percent of the 207 examined isolates of *E. coli* were resistant to at least one antimicrobial. Several patterns emerged from this research, suggesting that when a bacterium acquires resistance to one antimicrobial it is likely to become resistant to others because of the transfer of mobile genetic elements that harbor regions of multiple drug resistance. This suggests that even with careful restriction of antimicrobial use on farms, bacteria may still pick up resistance unrelated to the antimicrobials being used.

Industrial food animal production, antimicrobial resistance, and human health. E.K. Silbergeld, J. Graham and L.B. Price. *Annual Review of Public Health*, 2008. 29: 151-169.

Summary: Reviews the use of antimicrobials in agriculture and presents evidence for resistance stemming from their use in food animals. States that agricultural use of antibiotics can significantly shorten the useful life of these drugs, which are also used to treat disease in humans and animals. Suggests that estimates of nontherapeutic antibiotic use in agriculture fall between 60 percent and 80 percent of total antimicrobial production in the U.S. Concludes that "the use of antimicrobials for nontherapeutic purposes in agriculture is a major factor driving the emergence of antimicrobial resistance globally," and that "prudent public health policy thus indicates that nontherapeutic uses of antimicrobials in food animal production should stop."

Effect of subtherapeutic administration of antibiotics on the prevalence of antibiotic-resistant *Escherichia coli* bacteria in feedlot cattle. T.W. Alexander, L.J. Yanke, E. Topp, M.E. Olson, R.R. Read, D.W. Morck, and T.A. McAllister. *Applied and Environmental Microbiology,* 2008. 74(14): 4405-4416.

Summary: A study of *E. coli* resistance in feedlot cattle when they were administered a sub-therapeutic level of antibiotics. Cattle previously not treated with antibiotics were brought to a research feedlot where they were divided into groups each receiving a different regimen of sub-therapeutic antibiotics along with one group as a control not being treated. Cattle were fed two different diets during their treatments, one silage based diet and another grain based. Cattle tested before entering the feedlot (before starting sub-therapeutic treatment) were colonized with *E. coli* resistant to tetracycline (TET) at a rate greater than 40 percent, suggesting a colonization of TET resistant *E. coli* from birth (i.e. there is a high population of *E. coli* in circulation with TET resistance). Additionally the group fed chlortetracycline plus sulfamethazine (TET-SUL) showed an increased rate of TET resistance. A grain-based diet also appeared to increase not only the finding of *E. coli* but also increased the rate of finding TET resistant *E. coli*. Noted is that when antibiotic treatment was stopped for a period of about one to two months during each diet there was not a significant decline in the shedding of resistant *E. coli* except in the TET-SUL group where a slight decline was observed. However, upon starting treatment again the decline was reversed and prevalence of resistance continued to climb. The authors do note that in previous

Summary: The goal of the study was to understand how antibiotics and antibiotic resistance genes respond to different levels of manure management. Management practices studied were high intensity management and low intensity management. High intensity was defined as amending with alfalfa and dried leaves with regular watering and turning the manure to enhance degradation. Low intensity was defined as piling or windrowing manure, which then received no treatment. A small scale pilot study and a large scale study were performed in which the large scale study focused on feedlot cattle manure and dairy cattle manure. The authors found that high intensity was more successful increasing the rate at which antibiotics degrade but was not a significant factor in reducing levels of resistant genes. Feedlot manure had a significantly higher level of resistant genes than did dairy manure, likely due to the feedlot cattle receiving routine subtherapeutic concentrations of antibiotics. The persistence of resistant genes is speculated to be due to the presence of degraded antibiotics as these products are often still effective and may allow selection pressure to remain. The authors suggest that longer treatment times may be necessary to further reduce levels of antibiotic resistant genes.

Association of antimicrobial resistance in *Campylobacter* isolated from food-producing animals with antimicrobial use on farms. T. Asai, K. Harada, K. Ishihara, A. Kojima, T. Sameshima, Y. Tamura, and T. Takahashi. *Japanese Journal of Infectious Diseases*. 2007. 60: 290-294.

Summary: This study describes the use of antimicrobials in food-animals in Japan and examines the association between the use of antimicrobials and fluoroquinolone-resistant *Campylobacter*. Fluoroquinolone resistance was of interest because it was approved for therapeutic use in food-production animals in 1991. The most widely used antimicrobials were tetracyclines (7.8 percent), penicillin (6.5 percent), aminoglycosides (4.6 percent), and macrolides and lincosamides (4.3 percent). Fluoroquinolones were used for therapeutic purposes on 1.5 percent of 1,374 operations surveyed. Of operations positive for *C. jejuni*, oxytetracycline (OTC) resistance was present in 57.1 percent of operations using tetracycline antibiotics and on 43.2 percent of operations not using tetracyclines. For *C. coli* positive operations, 92.5 percent using tetracylcines and 74.3 percent not using tetracyclines were resistant to OTC. Enrofloxacin (ERFX) resistance was found in 66.7 percent and 16.7 percent farms reporting fluoroquinolone use for *C. jejuni* and *C. coli*. Farms not reporting use of fluoroquinolones had 15.5 percent and 28.8 percent prevalence of ERFX-resistant *C. jejuni* and *C. coli* respectively. Authors conclude that although fluoroquinolone-resistance in *Campylobacter* arose after approval for use in the treatment of sick animals, ERFX-resistance in *Campylobacter* is able to persist on food-animals operations regardless of use of fluoroquinolones.

Antibiotic resistance in bacteria associated with food animals: A United States perspective of livestock production. A.G. Mathew, R. Cissell, and S. Liamthong. *Foodborne Pathogens and Disease*. 2007. 4(2): 115-133.

Summary: Reviews the debate concerning use of antibiotics in animal production in the U.S. by presenting benefits of use but focusing on the problem of development of antibiotic resistant bacteria. Presents the types of antibiotics used in food-animal production and the main reasons for use including therapeutic, metaphylaxis (short-term treatment of infection), prophylactic, and for growth promotion. The origin of antibiotic resistance and the mechanisms of development and transfer of resistance among bacteria are discussed in detail. Surveillance programs are now present in the U.S. to track resistant bacteria and three are discussed. Based on surveillance and prior research, *Salmonella, Campylobacter, Listeria, Yersinia, Enterococcus,* and *Escherichia coli*

Concerned Scientists per-animal estimates of antibiotic feed-additive use for certain animal groups. North Carolina and Iowa both use an estimated three million pounds of antibiotics annually, which is equal to the estimated amount used for human treatment nationwide. The highest amounts of medically important antibiotics are used in hogs. An estimated 13.5 million pounds of antibiotics are excreted in the form of animal wastes, which is nearly half of the estimated total amount added to animal feeds. Also highlights that food is a pathway for resistance gene spread and that disease such as urinary tract infections may originate from food sources.

The potential role of concentrated animal feeding operations in infectious disease epidemics and antibiotic resistance. M.J. Gilchrist, C. Greko, D.B. Wallinga, G.W. Beran, D.R. Riley and P.S. Thorne. *Environmental Health Perspectives*, 2007. 115(2): 313-316.

Summary: Reports the recommendations of a working group that was part of the 2005 *"Conference on Environmental Health Impacts of Concentrated Animal Feeding Operations: Anticipating Hazards – Searching for Solutions."* Recommendations include the following: discontinue nontherapeutic use of antibiotics as growth promoters; establish nationwide surveillance programs to fully assess the contribution of antibiotic use in livestock production to the creation of ecological reservoirs of resistance or the transmission of that resistance to humans; identify resistant strains; and establish minimum separation distances for swine and poultry facilities to reduce the risk of influenza outbreaks and municipal-style waste treatment to limit microbial and nutrient contamination of surface and groundwater.

Fluoroquinolone-resistant *Campylobacter* species and the withdrawal of fluoroquinolones from use in poultry: A public health success story. J.M. Nelson, T.M. Chiller, J.H. Powers and F.J. Angulo. *Clinical Infectious Diseases,* 2007. 44: 977-980.

Summary: Reviews fluoroquinolone use and the resulting effect of resistance occurring in the *Campylobacter* that followed the withdrawal of enrofloxacin from use in treating poultry. States that 13 percent of all resistant infections occur from travel abroad, showing that resistance is a global threat and that U.S. regulatory actions are not effective internationally. Concludes that "judicious use of antimicrobial agents should be stressed to preserve the efficacy of these important chemotherapeutic agents."

Environmental health impacts of concentrated animal feeding operations: Anticipating hazards—searching for solutions. P.S. Thorne. *Environmental Health Perspective,* 2007. 115: 296-297.

Summary: Outlines potential risks to human health from concentrated animal feeding operations (CAFOs) and the research needed to better understand the impact of these operations on public health. Examples of policy change include establishment of a requirement for minimum separation distances, use of solid-waste storage tanks to eliminate the possibility of microbial contamination spreading to water sources and provision of clean water sources for drinking. Expresses concerns over air quality and the need for better surveillance in this area. Expresses a need to phase out the use of antimicrobial agents as growth promotants.

Response of antibiotics and resistance genes to high-intensity and low-intensity manure management. H.N. Storteboom, S-C Kim, K.C. Doesken, K.H. Carlson, J.G. Davis, and A. Pruden. *Journal of Environmental Quality,* 2007. 36: 1695-1703.

Summary: Reviews antimicrobial-resistant infections occurring in humans as a result of antibiotic use in food animal production. States that "a review of outbreaks of *Salmonella* infections indicated that outbreaks were more likely to have a food animal source than outbreaks caused by anti-microbial-susceptible *Salmonella.*" Reports that the human health consequences resulting from bacterial resistance include infections caused by resistant pathogens, an increase in treatment failures and increased severity of disease.

Nontherapeutic use of antimicrobial agents in animal agriculture: Implications for pediatrics. K.M. Shea. *Pediatrics,* 2004. 114(3): 862-868.

Summary: Examines how antimicrobials are used in food animal production and how this practice could contribute to resistance in humans. Notes that children are at greater risk from resistant infections than the general population.

Antibiotic use in agriculture and its impact on the terrestrial environment. K. Kumar, S.C. Gupta, Y. Chander and A.K. Singh. *Advances in Agronomy*, 2005. 87: 1-54.

Summary: Discusses the impact of antibiotic use on disease treatment and growth promotion in animals. States that overuse of antibiotics results in the excretion of drugs that are not absorbed in the animal and that the resulting manure stock may be spread on fields, altering the soil bacteria and contaminating water sources. Notes that the continued prevalent use of antibiotics in agriculture is increasing the emergence of antibiotic- resistant bacteria both in both clinically relevant strains of pathogens and in normal commensal microorganisms. Concludes that "prudent use of antibiotics to a bare minimum along with alternative methods that minimize development and proliferation of resistant bacteria need investigation."

Agricultural antibiotics and human health: Does antibiotic use in agriculture have a greater impact than hospital use? D.L. Smith, J. Dushoff and J.G. Morris, Jr. *PLoS Medicine,* 2005. 2(8): 731-735.

Summary: Reviews the emergence and spread of antibiotic-resistant bacteria and notes that mathematical models can help with understanding underlying mechanisms and guiding policy responses. Agricultural antibiotic use may generate novel types of antibiotic-resistant bacteria that spread to humans; models can help estimate how much additional disease has been caused by agricultural antibiotic use. Depending on the assumptions used, the model suggests that transmission from agriculture can have a greater impact than hospital transmission on human populations.

Resistant bugs and antibiotic drugs – State and county estimates of antibiotics in agricultural feed and animal waste. K. Florini, R. Denison, T. Stiffler, T. Fitzgerald, and R. Goldburg. *Environmental Defense,* 2005.

Summary: A report on the use of antibiotics in food animal production. States that an estimated 70 percent of the antibiotics used in the U.S. each year are used as feed additives for chickens, hogs and beef cattle. These are used mainly to promote growth and to compensate for poor health conditions. The National Academy of Sciences estimates that a cost estimate of $4 to $5 billion is associated with antibiotic-resistant bacteria. The report presents state and county specific estimates of antibiotic use and estimates of the amount of antibiotics excreted as animal waste. Farm families and the surrounding communities where there is greater on-farm antibiotic use may be at a greater risk of exposure to resistant bacteria. Estimates were derived from the U.S. Department of Agriculture's 2002 Census of Agriculture in conjunction with the Union of

percent) were STEC 0157. The greatest prevalence of STEC was found in isolates from cattle, followed by humans, food, and swine. Though swine had the smallest prevalence of STEC, isolates from swine demonstrated the highest prevalence of resistance. Prevalence of resistance to ampicillin, sulfamethazole, gentamicin, tetracycline, and trimethoprim-sulfamethoxazole was greater in non-STEC isolates than STEC isolates, however, all isolates were susceptible to ceftriaxone and ceftiofur. Of 191 isolates identified as *E. coli* O157:H7, cattle remained the most frequent source, followed by humans, and food. No O157:H7 was found in swine samples. All isolates from food were susceptible to all antimicrobials tested. Isolates from humans and cattle demonstrated similar resistance prevalence to ampicillin (5 vs. 1 percent), cephalothin (4 vs. 1 percent), chloramphenicol (0 vs. 1 percent), sulfamethoxazole (9 vs. 12 percent), tetracycline (7 vs. 11 percent), and amoxicillin-clavulanic acid (0 vs. 1 percent). Antimicrobial treatment of *E. coli* O157:H7 infection in humans may lead to release of Shiga toxin leading to hemolytic uremic syndrome (HUS). However, clinical trials are underway in which a chemically synthesized analog of Shiga toxin receptor Gb3 is given to patients to absorb the toxin and prevent HUS. If these trials prove successful, antimicrobials may become more important in the treatment of infection from *E. coli* 0157:H7. The findings from this study that most STEC isolates were susceptible to all antimicrobials tested is encouraging. However, presence of resistance in isolates from swine and cattle to drugs used in these food-animals suggests that antimicrobial use in these animals contributes to the emergence of resistance in *E. coli* O157:H7.

Antimicrobial resistance in livestock. B. Catry, H. Laevens, L.A. Devriese, G. Opsomer and A. Kruif. *Journal of Veterinary Pharmacology and Therapeutics,* 2003. 26: 81-93.

Summary: Reviews resistance in animals from a veterinary perspective. Notes that resistance could result in economic losses and animal welfare problems for livestock producers and that "the resistance level in a population is directly related to amount of antimicrobial drugs used." States that commensal bacteria in healthy animals fed or administered antibiotics contain resistance genes that if ingested by humans could colonize the gut and transfer these genes to pathogenic bacteria. This transfer would result in treatment difficulty because of antibiotic resistance.

Emergence of multidrug-resistant *Salmonella enterica* Serotype Newport infections resistant to expanded-spectrum cephalosporins in the United States. A. Gupta, J. Fontana, C. Crowe, B. Bolstorff, A. Stout, S. Van Duyne, M.P. Hoekstra, J.M. Whichard, T.J. Barrett and F.J. Angulo. *Journal of Infectious Diseases,* 2003. 188: 1707-1716.

Summary: Discusses the emergence of new strains of multidrug-resistant *Salmonella* in New England. Reports that isolates of Newport-MDRAmpC among *Salmonella* serotype Newport from humans rose from 0 percent in 1998 to 53 percent in 2001. This strain shows resistance to amoxicillin/clavulanic acid, cephalothin, cefoxitin and ceftiofur. Concludes that the use of antimicrobial agents in livestock is linked to the emergence of antimicrobial-resistant nontyphoidal *Salmonella* and that the emergence of Newport-MDRAmpC strains in humans has coincided with the same infections in cattle.

Evidence of an association between use of anti-microbial agents in food animals and antimicrobial resistance among bacteria isolated from humans and the human health consequences of such resistance. F.J. Angulo, V.N. Nargund and T.C. Chiller. *Journal of Veterinary Medicine,* 2004. 51: 374-379.