I. Introduction

In an effort to prospectively monitor the emergence of antimicrobial resistance in zoonotic pathogens, the National Antimicrobial Resistance Monitoring System (NARMS) was established in 1996 by the Food and Drug Administration's Center for Veterinary Medicine in collaboration with the Centers for Disease Control and Prevention, and the United States Department of Agriculture (USDA).

The animal component of NARMS is housed within the Bacterial Epidemiology and Antimicrobial Resistance Research Unit (BEAR) of the USDA's Agricultural Research Service in Athens, Georgia. For this report, the animal component of NARMS comprises the testing of isolates obtained from food-producing animals at slaughter through the USDA Food Safety and Inspection Service (FSIS) Pathogen Reduction: Hazard Analysis and Critical Control Point (PR/HACCP) verification testing program.

The antimicrobial agents selected for study are representative of antimicrobials used in both human and veterinary medicine and are selected primarily based on therapeutic value although molecular mechanisms of resistance or treatment patterns may also influence selection. Non-Typhi *Salmonella* was chosen as a sentinel organism of the NARMS program. Testing of *Campylobacter* and *Escherichia coli* isolates from animals began in 1998 and 2000, respectively.

This report summarizes 2009 data for *Salmonella*, *Campylobacter*, and *E. coli* isolates from food-producing animals at slaughter (chicken, turkey, cattle, and swine). Resistance data for previous years is included; however, due to the amount of data and complexity of analyses involved, all permutations are not represented. Additional information on the animal component of NARMS including past annual reports, summary trend tables and graphs, as well as a component for interactive data analysis can be found on the USDA's NARMS web page (http://www.ars.usda.gov/saa/bear/narms). Other analyses are available upon request.

The 2008 NARMS Executive Report contains additional background information on sampling and testing methodology for the human and retail arms of NARMS as well as summary data from all three components.

II. Sampling and Testing Methods

A. Samples

The *Salmonella* isolates included in this report were recovered by FSIS from carcass rinsates (chickens), carcass swabs (turkeys, cattle, and swine), and ground products (chickens, turkeys, and beef). *Campylobacter* and *E. coli* isolates included in this report were recovered by BEAR from FSIS Eastern Lab carcass rinsates (chickens).

Sampling methods used by FSIS for the PR/HACCP *Salmonella* verification testing program have changed since NARMS animal testing began. Before June of 2006, there were two phases of the FSIS regulatory program for *Salmonella* in raw products: non-targeted and targeted testing. Non-targeted samples were collected randomly from eligible federally inspected establishments, with a goal of scheduling every eligible establishment at least once a year. Targeted samples were collected from establishments that had a previously failed sample set. Beginning in June of 2006, sampling was scheduled using risk-based criteria designed to focus FSIS resources on establishments with the most samples positive for *Salmonella* and the greatest number of samples with serotypes most frequently associated with human salmonellosis[1,2]. Once the establishments presenting the greatest risk are sampled, FSIS prioritizes sampling at the establishments that have not been sampled within the last two years.

B. Isolation and Identification

1. *Salmonella*: Isolation from slaughter samples was conducted by FSIS at all three FSIS Regulatory Field Services Laboratories [Eastern (Athens, GA), Midwestern (St. Louis, MO) and Western (Alameda, CA)] following the "Isolation and Identification of *Salmonella* from Meat, Poultry, and Egg" procedures as described in the Microbiology Laboratory Guidebook, section 4[3,4]. Each FSIS laboratory processes samples collected throughout the U.S. Isolates were forwarded by FSIS to the National Veterinary Services Laboratories, Ames, IA (NVSL) for serotyping and a duplicate isolate was sent to BEAR for susceptibility testing and Pulsed Field Gel Electrophoresis (PFGE). Serotype results were subsequently sent to the BEAR unit as they became available.

2. *Campylobacter*: From 1998 to 2000, *Campylobacter* was isolated by all FSIS laboratories as part of the chicken monitoring baseline programs using the method described in the FSIS Microbiology Laboratory Guidebook[5]. Following presumptive identification, isolates were sent to BEAR for final confirmation and susceptibility testing as described below. Upon review of susceptibility data and isolation methods, it was determined that use of nalidixic acid as part of the culture selection criteria may have resulted in recovery of isolates more likely to be resistant to quinolones. A comparative study was initiated by BEAR in 2001.

For the first half of 2001, BEAR pilot tested several isolation methods for *Campylobacter* prior to adopting a new method in July. Since that time, only rinsates from the FSIS Eastern Lab containing $\geq$ 10 ml have been used. Thus, all rinsates tested for *Salmonella* were not processed for *Campylobacter* or *E. coli*. Also important to note is that when the FSIS *Campylobacter* baseline testing ended, rinsates were

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[1] USDA/FSIS. 2008. Serotypes Profile of Salmonella Isolates from Meat and Poultry Products. Available at http://www.fsis.usda.gov/Science/Serotypes_Profile_Salmonella_Isolates/index.asp.

[2] USDA/FSIS. FSIS Scheduling Criteria for Salmonella Sets in Raw Classes of Product. Available at http://www.fsis.usda.gov/PDF/Scheduling_Criteria_Salmonella_Sets.pdf.

[3] USDA/FSIS. 2004. Isolation and Identification of *Salmonella* from Meat, Poultry, and Egg Products. Microbiological Lab Guidebook 4.03. Available at http://www.fsis.usda.gov/PDF/MLG_4_03.pdf.

[4] USDA/FSIS. 2010. Laboratories and Procedures. Available a.t http://www.fsis.usda.gov/Science/Laboratories_&_Procedures/index.asp.

[5] USDA/FSIS. 1998. Isolation, Identification, And Enumeration Of Campylobacter jejuni/coli From Meat And Poultry Products. Microbiology Laboratory Guidebook, chapter 6. Available at http://www.fsis.usda.gov/ophs/Microlab/Mlgchp6.pdf.

no longer temperature controlled during shipment which may have affected isolate recovery. For *Campylobacter* isolation, 10 mls of rinsate was enriched in an equal volume of Campylobacter Enrichment Broth without blood under microaerobic conditions for 48 h at 42°C. Aliquots were struck onto Campy Cefex agar and plates were incubated as above. Final confirmation and speciation of *Campylobacter* isolates were obtained using the BAX® System Q7 (DuPont Qualicon; Wilmington, DE). This real-time PCR assay, able to detect *C. coli*, *C. jejuni*, and *C. lari*, was performed according to manufacturer's directions.

3. *Escherichia coli*: BEAR started isolating generic *E. coli* from the same rinsates used for *Campylobacter* isolation in 2000. For *E. coli*, a sample of the rinsate was enriched overnight before streaking onto a CHROMAgar™ ECC plate (DRG International; Mountainside, NJ). Plates were incubated at 36°C ± 1°C for 18-24 h as described by the manufacturer. Blue-green colonies, typical of generic *E. coli,* were selected for susceptibility testing and confirmed as *E. coli* using the Vitek (bioMérieux, Inc; Durham, NC).

C. Antimicrobial Susceptibility

In 2009, *Salmonella*, *Campylobacter*, and *E. coli* were tested using a semi-automated broth micro dilution system (Sensitire®, Trek Diagnostic Systems, Inc., Westlake, Ohio) and a custom made 96-well panel of antimicrobials (catalog no. CMV1AGNF for *Salmonella* and *E. coli;* catalog no. CAMPY for *Campylobacter*) to determine the minimum inhibitory concentration (MIC) of antimicrobials important in both human and veterinary medicine. Tables 1 and 2 list the antimicrobials tested, including the breakpoints for *Salmonella*/*E. coli* and *Campylobacter*, respectively. From 1998-2004, MICs for *Campylobacter* isolates were determined using Etest® (AB Biodisk; Solna, Sweden) as per manufacturer's direction with the exception that MICs were not rounded up prior to categorization. In 2005, the animal arm of NARMS switched to using the Sensititre® broth microdilution system for *Campylobacter* although the antimicrobials tested as described above for *Salmonella* and *E. coli* differed (Table 2). Regardless of the susceptibility testing method used, antimicrobial resistance was determined using Clinical and Laboratory Standards Institute (CLSI) breakpoints, when available[6,7,8].

In January 2010, CLSI published new MIC breakpoints for several cephalosporin antimicrobials for Enterobacteriaceae[9]. In particular, the resistance breakpoint for ceftriaxone changed (decreased) from ≥ 64 µg/ml to ≥ 4 µg/ml. In this report, the revised breakpoints for ceftriaxone are used and have been retrospectively applied to data from previous years; therefore, ceftriaxone resistance in previous reports will differ from what is presented in this report. It is important to note that the actual raw data has not changed over time, only the way that it is interpreted. For antimicrobial agents without CLSI approved breakpoints, interpretive criteria established by the NARMS working group were used.

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[6] CLSI. 2006. Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Approved Guideline. CLSI document M45-A. CLSI, Wayne, PA.
[7] CLSI. 2008. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard—Third Edition. CLSI document M31-A3. CLSI, Wayne, PA.
[8] CLSI. 2009. Performance Standards for Antimicrobial Susceptibility Testing; Nineteenth Informational Supplement. CLSI document M100-S19. CLSI, Wayne, PA.
[9] CLSI. 2010. Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement. CLSI document M100-S20. CLSI, Wayne, PA.

Quality control strains used for *Salmonella* and *E. coli* susceptibility testing included *E. coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 29213. *Campylobacter jejuni* ATCC 33560 was used as a control for *Campylobacter* susceptibility testing.

Table 1. *Salmonella* and *E. coli* Interpretive Criteria (breakpoints)[10]

CLSI Antimicrobial Class[11]	Antimicrobial Agent	Breakpoints (µg/ml)		
		Susceptible	Intermediate	Resistant
Aminoglycosides	Amikacin	≤ 16	32	≥ 64
	Gentamicin	≤ 4	8	≥ 16
	Kanamycin	≤ 16	32	≥ 64
	Streptomycin[12]	≤ 32	Not Applicable	≥ 64
β-Lactam/β-Lactamase Inhibitor Combinations	Amoxicillin–Clavulanic Acid	≤ 8 / 4	16/8	≥ 32 / 16
Cephems	Cefoxitin	≤ 8	16	≥ 32
	Ceftiofur	≤ 2	4	≥ 8
	Ceftriaxone[13]	≤ 1	2	≥ 4
	Cephalothin	≤ 8	16	≥ 32
Folate Pathway Inhibitors	Sulfonamides[14]	≤ 256	Not Applicable	≥ 512
	Trimethoprim–Sulfamethoxazole	≤ 2 / 38	Not Applicable	≥ 4 / 76
Penicillins	Ampicillin	≤ 8	16	≥ 32
Phenicols	Chloramphenicol	≤ 8	16	≥ 32
Quinolones	Ciprofloxacin	≤ 1	2	≥ 4
	Nalidixic acid	≤ 16	Not Applicable	≥ 32
Tetracyclines	Tetracycline	≤ 4	8	≥ 16

[10] Breakpoints established by CLSI (Clinical and Laboratory Standards Institute) were used when available
[11] According to CLSI M100 document
[12] There are no CLSI breakpoints for streptomycin
[13] In this report, the revised ceftriaxone breakpoints from the CLSI M100-S20 document, published in January 2010, were used (≥ 4 µg/ml). In previous NARMS reports the ceftriaxone breakpoints from the CLSI M100-S19 were used (≥ 64 µg/ml)
[14] From 1997 through 2003, sulfamethoxazole was tested. Sulfisoxazole replaced sulfamethoxazole beginning in 2004

Table 2. *Campylobacter* Interpretive Criteria (breakpoints)[15]

CLSI Antimicrobial Class[16]	Antimicrobial Agent	Breakpoints (µg/ml) Etest (1998-2004)			Breakpoints (µg/ml) Broth Microdilution (2005-2009)		
		Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
Aminoglycosides	Gentamicin	≤ 4	8	≥ 16	≤ 2	4	≥ 8
Lincosamides	Clindamicin	≤ 0.5	1 - 2	≥ 4	≤ 2	4	≥ 8
Macrolides	Azithromycin	≤ 0.25	0.5 - 1	≥ 2	≤ 2	4	≥ 8
	Erythromycin	≤ 0.5	1 - 4	≥ 8	≤ 8	16	≥ 32
Ketolides	Telithromycin	Not Tested	Not Tested	Not Tested	≤ 4	8	≥ 16
Phenicols	Florfenicol	Not Tested	Not Tested	Not Tested	≤ 4	Not Applicable	Not Applicable
	Chloramphenicol	≤ 8	16	≥ 32	Not Tested	Not Tested	Not Tested
Fluoroquinolones	Ciprofloxacin	≤ 1	2	≥ 4	≤ 1	2	≥ 4
Quinolones	Nalidixic acid	≤ 16	Not Applicable	≥ 32	≤ 16	32	≥ 64
Tetracyclines	Tetracycline	≤ 4	8	≥ 16	≤ 4	8	≥ 16

[15] Breakpoints established by CLSI (Clinical and Laboratory Standards Institute) were used when available. CLSI breakpoints are available only for erythromycin, ciprofloxacin, and tetracycline

[16] According to CLSI M100 document

D. Phage Typing

Salmonella Typhimurium and *S.* Typhimurium variant 5- isolates with resistance to at least ampicillin, chloramphenicol, sulfisoxazole and tetracycline (ACSuT) were submitted to NVSL for phage typing.

III. Reporting Methods

WHONET 5, a free microbiology laboratory database software program, was used to categorize MICs as resistant, intermediate (when applicable), and susceptible according to CLSI established interpretive criteria (when available). The 95% confidence interval was calculated using the Wilson interval with continuity correction method in WHONET 5. Resistance percentages by food animal source and organism are presented from 1997 through 2009 for *Salmonella*, from 1998 through 2009 for *Campylobacter*, and from 2000 through 2009 for *E. coli*. Additionally, MIC distributions are presented for 2009. For *Salmonella*, MIC distributions were tabulated on both macro and micro levels. At the macro level, all *Salmonella* serotypes were combined and analyzed for MIC distributions. At the micro level, isolates were grouped by serotype prior to analysis. Results were tabulated for the top serotypes from chickens, turkeys, cattle, and swine. MIC distributions were tabulated separately for *C. coli* and *C. jejuni*. The change of sample collection methods by FSIS in 2006 limits meaningful trend comparison between pre-2006 results and post-2006 results. Similarly, these changes limit year-to-year comparisons post-2006[17].

In this report, MDR is reported as resistance to more than one antimicrobial class (i.e. multiple antimicrobials may be included in a class and resistance to any one antimicrobial within a class results in the designation of the class being resistant).

The antimicrobial classes used for MDR tabulations for *Salmonella* and *E. coli* were aminoglycosides (amikacin, gentamicin, kanamycin and streptomycin), β-lactam/β-lactamase inhibitor combinations (amoxicillin-clavulanic acid), cephems (cefoxitin, ceftiofur and ceftriaxone), penicillins (ampicillin), folate pathway inhibitors (sulfonamides and trimethoprim/sulfamethoxazole), phenicols (chloramphenicol), quinolones (ciprofloxacin and nalidixic acid), and tetracyclines (tetracycline). The antimicrobial classes used for MDR tabulations for *Campylobacter* were aminoglycosides (gentamicin), ketolides (telithromycin 2005-2009), lincosamides (clindamycin), macrolides (azithromycin and erythromycin), phenicols (chloramphenicol 1998-2004 and florfenicol 2005-2009), quinolones (ciprofloxacin and nalidixic acid) and tetracyclines (tetracycline).

[17] USDA/FSIS. 2008. Serotypes Profile of Salmonella Isolates from Meat and Poultry Products. Available at http://www.fsis.usda.gov/Science/Serotypes_Profile_Salmonella_Isolates/index.asp.