II. Sampling and Testing Methods

A. Samples

Salmonella was recovered and tested across four animal species (chicken (broiler), turkey, cattle (beef and dairy), and swine). Isolates were received from FSIS as part of their regulatory testing. Information related to FSIS collection and testing methodology can be found at http://www.fsis.usda.gov/Science/Laboratories_&_Procedures/index.asp. FSIS progress reports on *Salmonella* testing of selected raw meat and poultry products from 2006 through present can be accessed at http://origin-www.fsis.usda.gov/Science/Quarterly_*Salmonella*_Results/index.asp.

B. Isolation

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.[1] Isolates were forwarded by FSIS to National Veterinary Services Laboratories, Ames, IA (NVSL) for serotyping. Serotype results were subsequently sent to the BEAR unit as they became available.

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[2]. Upon 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* until settling on a new method that was adopted in July which involved concentrating spent carcass rinsate prior to culture. Since that time, only rinsates from the FSIS Eastern Lab containing $\geq$10 ml were used. Thus, all rinsates tested for *Salmonella* were not processed for *Campylobacter* or *E. coli*. Also important to note is that as the FSIS *Campylobacter* baseline testing stopped, rinsates were no longer temperature controlled during shipment.

BEAR started isolating generic *E. coli* from these same rinsates in 2000. For *E. coli*, a sample of the rinsate was pre-enriched overnight before streaking onto a CHROMAgar™ ECC plate (DRG International; Mountainside, NJ). Plates were incubated as described by the manufacturer. Blue-green colonies,

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[1] 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.

[2] 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.

typical of *E. coli,* were selected for susceptibility testing and confirmed as *E. coli* using the Vitek (bioMérieux, Inc; Durham, NC).

C. *Campylobacter* Identification

Final confirmation and speciation of *Campylobacter* isolates were obtained using the *Campylobacter* BAX® PCR System (DuPont Qualicon; Wilmington, DE). This multiplex assay, specific for *C. coli* and *C. jejuni*, was performed according to manufacturer's directions as previously described.[3]

D. Antimicrobial Susceptibility

In 2007, *Salmonella*, *Campylobacter*, and *E. coli* were tested using a semi-automated system (Sensitire®, Trek Diagnostic Systems, Inc., Westlake, Ohio) and a custom panel (catalog no. CMV1AGNF for *Salmonella* and *E. coli;* catalog no. CAMPY for *Campylobacter*) to determine the minimum inhibitory concentration (MIC) to antimicrobials important in both human and veterinary medicine. Tables 1 and 2 list antimicrobials tested, including their 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, except MICs were not rounded up prior to categorization. In 2005, the animal component of NARMS switched to broth microdilution using the Sensititre system for *Campylobacter* as described above for *Salmonella* and *E. coli*. Regardless of the susceptibility testing method used, antimicrobial resistance was determined using Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) breakpoints, when available.[4,5] For antimicrobial agents without CLSI approved breakpoints, interpretive criteria as established by the NARMS working group were used.

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

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[3] Englen, M.D. and Paula 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.

[4] NCCLS/CLSI. 2002. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals. Approved Standard, M31-A2. NCCLS, Wayne, PA.

[5] CLSI. 2006. Performance Standards for Antimicrobial Susceptibility Testing; Sixteenth Informational Supplement (M100-S16). CLSI, Wayne, PA.

Table 1. Breakpoints Used for Susceptibility Testing of *Salmonella* and *E. coli*[1]

CLSI Subclass[2]	Antimicrobial Agent	Breakpoints (µg/ml)		
		Susceptible	Intermediate	Resistant
Aminoglycosides	Amikacin	≤ 16	32	≥ 64
	Gentamicin	≤ 4	8	≥ 16
	Kanamycin	≤ 16	32	≥ 64
	Streptomycin[3]	≤ 32	Not Applicable	≥ 64
Aminopenicillins	Ampicillin	≤ 8	16	≥ 32
β-Lactam/β-Lactamase Inhibitor Combinations	Amoxicillin–Clavulanic Acid	≤ 8 / 4	16/8	≥ 32 / 16
Cephalosporins I	Cephalothin	≤ 8	16	≥ 32
Cephalosporins III	Ceftiofur	≤ 2	4	≥ 8
	Ceftriaxone	≤ 8	16 - 32	≥ 64
Cephamycins	Cefoxitin	≤ 8	16	≥ 32
Folate Pathway Inhibitors	Sulfonamides[4]	≤ 256	Not Applicable	≥ 512
	Trimethoprim–Sulfamethoxazole	≤ 2 / 38	Not Applicable	≥ 4 / 76
Phenicols	Chloramphenicol	≤ 8	16	≥ 32
Fluoroquinolones	Ciprofloxacin	≤ 1	2	≥ 4
Quinolones	Nalidixic acid	≤ 16	Not Applicable	≥ 32
Tetracyclines	Tetracycline	≤ 4	8	≥ 16

[1] Breakpoints established by CLSI (Clinical and Laboratory Standards Institute) were used when available

[2] According to CLSI M100 document

[3] There are no CLSI breakpoints for streptomycin

[4] From 1997 through 2003, sulfamethoxazole was tested. Sulfisoxazole replaced sulfamethoxazole beginning in 2004

Table 2. Breakpoints Used for Susceptibility Testing of *Campylobacter*[1]

CLSI Subclass[2]	Antimicrobial Agent	Breakpoints (µg/ml) Etest (1998-2004)			Breakpoints (µg/ml) Broth Microdilution (2005-2007)		
		Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
Aminoglycosides	Gentamicin	≤ 4	8	≥ 16	≤ 2	4	≥ 8
Lincosamides	Clindamycin	≤ 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

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

[2] According to CLSI M100 document

E. Phage Typing

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

III. Reporting Methods

WHONET 5, a microbiology laboratory database software, was used to categorize MICs as resistant, intermediate susceptibility (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. MIC distributions as well as resistance and intermediate susceptibility percentages were tabulated by pathogen and food animal source. For *Salmonella*, MIC distributions were tabulated both on macro and micro levels. At the macro level, all *Salmonella* were analyzed for MIC distributions. At the micro level, isolates were grouped by serotype prior to analysis; results were tabulated for the top 11 serotypes from chicken, cattle and swine and for the top eight serotypes from turkey. MIC distributions were tabulated separately for *C. coli* and *C. jejuni*. Additionally, historical resistance percentages by food animal source and organism are presented from 1997 through 2007 for *Salmonella*, from 1998 through 2007 for *Campylobacter*, and from 2000 through 2007 for *E. coli*.

The frequency of *S.* Typhimurium showing resistance to at least ACSSuT (ampicillin, chloramphenicol, streptomycin, sulfisoxazole and tetracycline) or ACSuT (ampicillin, chloramphenicol, sulfisoxazole and tetracycline) as well as phage type distributions are reported separately for *S.* Typhimurium and *S.* Typhimurium variant 5- isolates. The frequency and percentage of confirmed *S.* Typhimurium DT104 isolates is reported separately by food animal source from 1997 through 2007.

Previously, multiple drug resistance (MDR) was defined as resistance to two or more antimicrobials regardless of subclass. In this report, MDR is reported as resistance to more than one CLSI subclass.

MDR tabulations for all pathogens were limited to only those antimicrobials tested for all years. The 14 core antimicrobials for *Salmonella* and *E. coli* were amikacin, gentamicin, kanamycin, streptomycin, ampicillin, amoxicillin/clavulanic acid, ceftiofur, ceftriaxone, chloramphenicol, sulfonamides (sulfamthoxazole/sulfisoxazole), trimethoprim/sulfamethoxazole, ciprofloxacin, nalidixic acid, and tetracycline. The seven core antimicrobials for *Campylobacter* were gentamicin, clindamycin, azithromycin, erythromycin, ciprofloxacin, nalidixic acid and tetracycline.