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I. Introduction

In an effort to prospectively monitor the occurrence of antimicrobial resistance of zoonotic pathogens from human diagnostic specimens, retail meats and food animals, 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 Center for Disease Control and Prevention (CDC), 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 Agricultural Research Service (ARS) in Athens, Georgia. The animal component of NARMS comprises the testing of isolates obtained from diagnostic animal specimens, healthy on-farm animals, and food-producing animals at slaughter. The panel of antimicrobial agents chosen is representative of common antimicrobials used in both human and veterinary medicine. Non-typhoid *Salmonella* was chosen as a sentinel organism of the animal component of NARMS which was launched in 1997. Testing of Campylobacter isolates began in 1998 while Escherichia coli was included in 2000. In 2004, Enterococcus data was added.

This report summarizes 2006 data for *Salmonella*, *Campylobacter*, *E.coli*, and *Enterococcus* isolates from food-producing animals at slaughter (carcass rinsates, carcass swabs, and ground products) obtained through USDA's Food Safety and Inspection Service (FSIS) Pathogen Reduction: Hazard Analysis and Critical Control Point (PR/HACCP) verification testing program. When suitable, resistance trends are also 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 can be found on the web at http://www.ars.usda.gov/Main/docs.htm?docid=14491.

The 2003 NARMS Executive Report also contains additional background information on sampling and testing methodology as well as summary data from all three components of the program and is available on the web at http://www.fda.gov/cvm/Documents/NARMSExecSum03.pdf. At the time of this posting, the 2004 NARMS Executive Report was near completion, the link to this report will be available here.

II. Sampling and Testing Methods

A. Samples

Salmonella isolates were recovered from food animals at slaughter: carcass rinsates (chicken), carcass swabs (turkey, cattle and swine), and ground products (chicken, turkey, and beef) collected through USDA-FSIS's *Salmonella* PR/HACCP verification testing program from all federally inspected plants throughout the United States. Recovery of *Campylobacter*, *E. coli*, and *Enterococcus* was only attempted from chicken carcass rinsates. For this report, descriptions of isolates are confined by major animal species.

B. Isolation

Salmonella isolation from slaughter samples was conducted at all three FSIS Regulatory Field Services Laboratories (Eastern, Midwestern and Western) following the Isolation and Identification of *Salmonella* from Meat, Poultry, and Egg procedures as described in the Microbiology Laboratory Guidebook, section 4.[1] Positive isolates were forwarded by FSIS to the National Veterinary Services Laboratories (NVSL) for serotyping and were subsequently sent to the BEAR unit as serotyping results became available.

From 1998 to 2000, *Campylobacter* was isolated by FSIS using the method described in the FSIS Microbiology Laboratory Guidebook[2]. For the first half of 2001, ARS tested several isolation methods until

a new method was adopted in July. Since that time, *Campylobacter* has been isolated by ARS from FSIS' Eastern lab spent chicken carcass rinsates. ARS started isolating *E.coli* and *Enterococcus* from these same rinsates in 2000 and 2003, respectively. In 2003, all *Enterococcus* isolates obtained were tested for susceptibility. Beginning in 2004, a subset of *Enterococcus* isolates were selected for susceptibility testing with a maximum of 1,500 isolates tested each year. A total of 375 isolates were selected for each yearly quarter by selecting isolates from samples which also tested positive for *Salmonella*, *E.coli* and *Enterococcus*. Additionally, all odd *Enterococcus* species found (*avium, cecorum, malodoratus*, and *gilvus*) were tested. Any remaining isolates to test were selected by selecting 30% *E. faecalis*, 30% *E. faecium*, 10% *E. durans*, 10% *E. hirae*, 10% *E. casseliflavus*, and 10% *E. gallinarum*.

Additionally, *Enterococcus* and *Campylobacter* speciation was also performed as described below.

C. *Enterococcus* Speciation

A species-specific multiplex PCR was performed on presumptive *Enterococcus* isolates which provided a simultaneous genus and species identification of 23 species of enterococci.[3] Confirmed *Enterococcus* isolates of other species not identified with this procedure were labeled as '*Enterococcus* species'.

D. *Campylobacter* Speciation

Final confirmation and speciation were obtained using the *Campylobacter* BAX® PCR (DuPont Qualicon; Wilmington, DE). This multiplex assay, specific for *C. coli* and *C. jejuni*, was performed according to manufacturer's directions as previously described[4].

E. Antimicrobial Susceptibility

Salmonella, *Campylobacter*, *E.coli*, and *Enterococcus* were tested using a semi-automated system (Sensitire®, Trek Diagnostic Systems, Westlake, Ohio). Resistance trends for *Campylobacte*r include data from 1998-2004 which was obtained using Etest® (AB Biodisk). Antimicrobial resistance was determined using Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) standards, when available.[5,6] For antimicrobial agents without CLSI approved standards, NARMS interpretive criteria as established by the NARMS working group were used. Tables 1, 2 and 3 list antimicrobials tested and their breakpoints for *Salmonella/E.coli*, *Campylobacter*, and *Enterococcus* respectively.

Quality control strains used for *Salmonella* and *E.coli* included *E.coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, and *Staphylococcus aureus* ATCC 29213. *Campylobacter jejuni* ATCC 33560 was used for *Campylobacter* testing while four strains were used for testing *Enterococcus*: *Enterococcus faecalis* ATCC 29212, *Enterococcus faecalis* ATCC 51299, *E.coli* ATCC 25922, and *Staphylococcus aureus* ATCC 29213.

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

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[3] Jackson, C. 2004. Use of a Genus- and Species-Specific Multiplex PCR for Identification of *Enterococci*. Journal of Clinical Microbiology, *42*(8):3558-65.

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

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