Introduction to United States Department of Agriculture VetNet: Status of *Salmonella* and *Campylobacter* Databases from 2004 Through 2005

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ABSTRACT

In 2003 the United States Department of Agriculture established USDA VetNet. It was modeled after PulseNet USA, the national molecular subtyping network for foodborne disease surveillance. The objectives of USDA VetNet are: to use pulsed-field gel electrophoresis (PFGE) to subtype zoonotic pathogens submitted to the animal arm of the National Antimicrobial Resistance Monitoring System (NARMS); examine VetNet and PulseNet PFGE patterns; and use the data for surveillance and investigation of suspected foodborne illness outbreaks. Whereas PulseNet subtypes 7 foodborne disease-causing bacteria—*Escherichia coli* O157:H7, *Salmonella*, *Shigella*, *Listeria monocytogenes*, *Campylobacter*, *Yersinia pestis*, and *Vibrio cholerae*—VetNet at present subtypes nontyphoidal *Salmonella* serotypes and *Campylobacter* from animals, including diagnostic specimens, healthy farm animals, and carcasses of food-producing animals at slaughter. By the end of 2005, VetNet had two functioning databases: the NARMS *Salmonella* and the NARMS *Campylobacter* databases. The *Salmonella* database contained 6763 *Salmonella* isolates and 2514 unique XbaI patterns, while the *Campylobacter* database contained 58 *Campylobacter* isolates and 53 unique Smal patterns. Both databases contain the PFGE tagged image file format (TIFF) images, demographic information, and the antimicrobial resistance profiles assigned by NARMS. In the future, veterinary diagnostic laboratories will be invited to participate in VetNet. The establishment of USDA VetNet enhances the mission of the agriculture and public health communities in the surveillance and investigation of foodborne illness outbreaks.

INTRODUCTION

Despite significant research efforts and ongoing surveillance, foodborne microbial pathogen outbreaks continue to occur. Furthermore, there is no resolution to the debate regarding the role of antimicrobial resistance in zoonotic pathogens in these outbreaks. Research designed to improve detection, identification, and characterization of foodborne pathogens and infectious disease outbreaks is required for development of prevention and mitigation programs for foodborne pathogens in the food supply.

Differentiation of bacterial isolates can use phenotypic and genotypic subtyping methods (Olive and Bean, 1999; Wiedmann, 2002). Subtyping organisms is primarily important in epi-

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demiologic studies for identification of disease outbreaks and the source of the outbreak or infection, but can be used for other purposes (Olive and Bean, 1999). The National Antimicrobial Resistance Monitoring System (NARMS) is a national program established in 1996 by the Food and Drug Administration (FDA), Centers for Disease Control and Prevention (CDC), and the United States Department of Agriculture (USDA) to monitor changes in antimicrobial susceptibility of enteric bacteria from human and animal sources (Fedorka-Cray et al., 2002; Marano et al., 2000). One goal of the animal arm of NARMS is to provide phenotypic analysis of potential foodborne pathogens with descriptive data on the extent and temporal trends of antimicrobial susceptibility in selected bacterial organisms from the animal population (Fedorka-Cray et al., 2002). At present, the NARMS program conducts susceptibility testing on nontyphoidal Salmonella, generic Escherichia coli, Campylobacter, and enterococci (Fedorka-Cray et al., 2002; Marano et al., 2000). Susceptibility testing of bacterial isolates provides valuable information on emerging resistance as well as for characterization of the isolates.

PulseNet USA is a national molecular subtyping network which provides surveillance for foodborne infections in the U.S. (Gerner-Smidt et al., 2006; Swaminathan et al., 2001) Although a number of molecular typing methods exist, pulsed-field gel electrophoresis (PFGE) has been termed the gold standard, and is the present method of choice by PulseNet for discriminating between epidemiologically related bacterial isolates (Birren et al., 1989; Lai et al., 1989; Olive and Bean, 1999; Swaminathan et al., 2001; Wiedmann, 2002). To that end, PFGE has been used successfully to detect bacterial outbreaks caused by Salmonella, E. coli, Campylobacter, Listeria, and Shigella (Barrett et al., 1994; Buchrieser et al., 1993; Olsen et al., 2001; Threlfall et al., 1996).

In 2003, to merge monitoring of antimicrobial resistance in bacterial organisms from animals with molecular subtyping surveillance using PFGE, the USDA Agricultural Research Service (ARS) initiated USDA VetNet. The goals of VetNet are to determine PFGE patterns of animal isolates collected from federally inspected slaughter and processing facilities submitted to NARMS; examine VetNet and PulseNet PFGE patterns; and use the data for surveillance and investigation of foodborne illness outbreaks. In 2004, the VetNet program began analyzing PFGE patterns of S. enterica isolates collected by the animal arm of NARMS and, in the following year, a NARMS Campylobacter database was created. Both databases contain isolates subtyped by PFGE according to standardized protocols developed by PulseNet. This report provides an introduction to USDA VetNet and summarizes the progress of the program as well as future endeavors.

MATERIALS AND METHODS

Origin of isolates

Bacterial isolates collected as part of the animal arm of NARMS originated from different sources and were classified as nondiagnostic or diagnostic (Fedorka-Cray et al., 2002). Nondiagnostic Salmonella isolates were collected by the Food Safety and Inspection Service (FSIS) from federally inspected slaughter and processing plants and were sent to ARS. In addition, ARS also received chicken carcass rinsates from the FSIS Eastern Laboratory which were then cultured for Campylobacter (Englen et al., 2003). Diagnostic isolates were collected from sentinel veterinary diagnostic laboratories and from the Animal and Plant Health Inspection Service (APHIS) National Veterinary Services Laboratories (NVSL) (Fedorka-Cray et al., 2002). Sentinel laboratories were chosen to augment the numbers of diagnostic isolates and to provide a geographic representation that complemented states submitting human isolates to the human arm of NARMS at the CDC. Salmonella and Campylobacter isolates from the sentinel laboratories originated from clinical specimens submitted to the laboratories as a result of clinical illness. Salmonella isolates from NVSL were associated with a primary or secondary disease outcome and originated from states without a sentinel laboratory, to avoid duplication. Bacteria were tested for antimicrobial susceptibility as previously described (Englen et al., 2005; Fedorka-Cray et al., 2002; Tankson et al., 2006).
Pulsed-field gel electrophoresis and pattern analysis

A 24–26 hour PFGE procedure was performed as described by PulseNet (Ribot et al., 2006; Ribot et al., 2001). All TIFF images were analyzed using BioNumerics v. 4.0 (Applied Maths Scientific Software Development, Sint-Martens-Latem, Belgium) and PulseNet masterscripts v. 2.0 according to PulseNet Standard Operating Procedures (CDC, 2000). Patterns were assigned by placing isolates in a comparison and arranging them by decreasing similarity. For Salmonella and Campylobacter, a position tolerance of 1.5% was used. Patterns were named using the PulseNet standardized method for pattern naming (CDC, 2000).

NARMS categories

Fifty states, the District of Columbia, Puerto Rico, Guam, and Mariana are divided into six NARMS regions: Region 1—Maine, Vermont, New Hampshire, New York, Massachusetts, Connecticut, Rhode Island, Pennsylvania, Maryland, Delaware, New Jersey, Ohio, Indiana, and Michigan; Region 2—Virginia, Kentucky, Tennessee, North Carolina, South Carolina, Georgia, Alabama, West Virginia, Florida, Washington D.C., and Puerto Rico; Region 3—North Dakota, South Dakota, Nebraska, Kansas, Minnesota, Iowa, Missouri, Wisconsin, and Illinois; Region 4—Oklahoma, Arkansas, Louisiana, Texas, and Mississippi; Region 5—Washington, Montana, Oregon, Idaho, Wyoming, Colorado, Utah, New Mexico, Arizona, Nevada, and California; and Region 6—Hawaii, Guam, Virgin Islands, and Mariana.

For this publication, isolate sources (host species) were combined to form larger groups. The groups were defined as follows: Bovine—cattle, bovine, beef cattle, cow/bull, dairy cattle, Holstein cattle, or steer/heifer; Canine—canine or dog; Chicken—broiler, chick, chicken, or young chicken; Beef—beef or ground beef; Porcine—market hog, porcine, potbellied pig, or swine; and Reptile—lizard, reptile, or snake.

In the NARMS Salmonella database, primary serotypes and their variants received the same three-character serotype code; therefore, the following variants were included with the primary serotype: Anatum—serotype Anatum or Anatum var. O 15+ (Newington); Choleraesuis—serotype Choleraesuis or Choleraesuis var. Kunzendorf; Give—serotype Give or Give var. O 15+ (Newbrunswick); Muenster—serotype Muenster or Muenster var. O 15+, 34+ (Arkansas); Orion—serotype Orion or Orion var. O 15+ (Binza); Paratyphi B—serotype Paratyphi B or Paratyphi B var. L(+) tartrate (Java); Typhimurium—serotype Typhimurium or Typhimurium var. O 5– (Copenhagen); and Uganda—serotype Uganda or Uganda var. O 15+ (Kinshasa).

RESULTS

NARMS Salmonella database

In May of 2004, the first PFGE TIFF images of Salmonella isolates were analyzed for inclusion in the NARMS Salmonella database. By the end of 2005, the database contained 6763 Salmonella PFGE patterns, with the date the isolate was frozen ranging from January 1, 1997 to December 22, 2005. For each isolate, the following information, if known, was stored in the database: serotype, subspecies, pattern number, country and region where the isolate was sampled, the type of sample (e.g., chicken, ground beef), the date the isolate was frozen, the date the PFGE plug was made, the date the PFGE gel was processed and which restriction enzyme was used, the date the PFGE gel was analyzed, the PFGE gel number and lane number where the isolate was run, the antigen formula, and the antimicrobial resistance profile.

The three most common Salmonella serotypes represented in the database were Newport (n = 1009; 14.9%), Typhimurium (n = 826; 12.2%), and Kentucky (n = 742; 11%) (Table 1). Although serotype Newport was the predominant serotype and serotype Agona was the fourth most common, these data may be overrepresented, as retrospective research studies were performed for these serotypes and the data were included in the Salmonella database. The majority of isolates (1880/6763; 28%) were from whole chicken sources, followed by bovine (n = 1470; 22%) and porcine (n = 1352; 20%) sources, including ground products (Table 2). S. enterica serotype Kentucky was the predominant serotype for both whole and
TABLE 1. NARMS Salmonella Database Top Serotypes

<table>
<thead>
<tr>
<th>Serotypea</th>
<th>No. of isolates (%)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newport</td>
<td>1009 (14.92)</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>826 (12.21)</td>
</tr>
<tr>
<td>Kentucky</td>
<td>742 (10.97)</td>
</tr>
<tr>
<td>Agona</td>
<td>617 (9.12)</td>
</tr>
<tr>
<td>Heidelberg</td>
<td>525 (7.76)</td>
</tr>
<tr>
<td>Enteritidis</td>
<td>293 (4.33)</td>
</tr>
<tr>
<td>Derby</td>
<td>229 (3.39)</td>
</tr>
<tr>
<td>Uganda</td>
<td>178 (2.63)</td>
</tr>
<tr>
<td>Anatum</td>
<td>172 (2.54)</td>
</tr>
<tr>
<td>Montevideo</td>
<td>161 (2.38)</td>
</tr>
</tbody>
</table>

aSerotypes Agona and Newport were involved in special projects; serotypes Anatum, Typhimurium, and Uganda included the primary serotype and variants.
bNumber/percent isolates out of total number of Salmonella isolates in database (\(n = 6763\)).

ground chicken, while serotype Newport was the predominant serotype for bovine, equine, and canine sources. Both serotype Heidelberg and Typhimurium were in the top three serotypes for four different sources, including whole and ground chicken and turkey for serotype Heidelberg and bovine, porcine, equine, and canine sources for serotype Typhimurium.

One hundred and sixteen S. enterica serotypes were represented in all six regions of NARMS. Included in this number were subspecies IIIa, IIIb, and IV; serotype variants were combined with the primary serotype. Regional data were available for approximately 94%
The number of isolates representing three NARMS regions (Regions 1, 2, and 3) were very close (Table 3). These three regions are quite large and contain 32 states, the District of Columbia, and Puerto Rico. Region 2 (VA, KY, TN, NC, SC, GA, AL, WV, FL, DC, and PR) had the most isolates (1634/6343; 26%), followed by Region 1 (1533; 24%) and Region 3 (1599; 25%). The top three Salmonella serotypes overall (Newport, Typhimurium, and Kentucky) were the predominant serotypes in Regions 1, 2, and 4. Salmonella serotype Typhimurium was one of the top three predominant serotypes in all six NARMS regions.

Of the 6763 Salmonella isolates, 2514 unique XbaI patterns have been identified. The three most common patterns were Kentucky pattern JGPX01.0003 (202; 3%), Kentucky pattern JGPX01.0001 (186; 2.8%), and Newport pattern JJPX01.0023 (180; 2.7%) (Fig. 1). Eighty percent of the top ten XbaI patterns in the database were represented by Salmonella serotypes Kentucky, Newport, Enteritidis, and Typhimurium.

Salmonella isolates were tested for resistance to various antimicrobials and the antimicrobial resistance profiles were entered into BioNumerics (isolates collected prior to 2004 may have been tested for resistance to different antimicrobials). Currently, isolates are tested for resistance to the following antimicrobials (resistance breakpoints in parentheses): amikacin (≥ 64 μg/mL), amoxicillin/clavulanic acid (≥ 32 μg/mL), ampicillin (≥ 32 μg/mL), cefoxitin (≥ 32 μg/mL), ceftiofur (≥ 8 μg/mL), ceftriaxone (≥ 64 μg/mL), chloramphenicol (≥ 32 μg/mL), ciprofloxacin (≥ 4 μg/mL), gentamicin (≥ 16 μg/mL), kanamycin (≥ 64 μg/mL), nalidixic acid (≥ 32 μg/mL), streptomycin (≥ 64 μg/mL), sulfamethoxazole (≥ 512 μg/mL), tetracycline (≥ 16 μg/mL), and trimethoprim/sulfamethoxazole (≥ 4/76 μg/mL). The database currently contains the antimicrobial resistance profiles for approximately 40% of the isolates analyzed by December 31, 2005; however, the susceptibility data for every isolate is available at www.ars.usda.gov/main/site_main.htm?modecode =66-12-05-08.

### Table 3. NARMS Salmonella Database Predominant Serotypes Per NARMS Regions

<table>
<thead>
<tr>
<th>Regiona</th>
<th>No. of isolates (%)b</th>
<th>Predominant serotypesc</th>
<th>No. per region (%)d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region 1 (ME, VT, NH, NY, MA, CT, RI, PA, MD, DE, NJ, OH, IN, MI)</td>
<td>1533 (24.17)</td>
<td>Newport</td>
<td>390 (25.44)</td>
</tr>
<tr>
<td>Region 2 (VA, KY, TN, NC, SC, GA, AL, WV, FL, DC, PR)</td>
<td>1634 (25.76)</td>
<td>Kentucky</td>
<td>190 (12.39)</td>
</tr>
<tr>
<td>Region 3 (ND, SD, NE, KS, MN, IA, MO, WI, IL)</td>
<td>1599 (25.21)</td>
<td>Typhimurium</td>
<td>189 (12.33)</td>
</tr>
<tr>
<td>Region 4 (OK, AR, LA, TX, MS)</td>
<td>818 (12.90)</td>
<td>Kentucky</td>
<td>275 (16.83)</td>
</tr>
<tr>
<td>Region 5 (WA, MT, OR, ID, WY, CO, UT, NM, AZ, NV, CA)</td>
<td>740 (11.67)</td>
<td>Typhimurium</td>
<td>149 (9.12)</td>
</tr>
<tr>
<td>Region 6 (HI, Guam, VI, Mariana)</td>
<td>19 (0.30)</td>
<td>Newport</td>
<td>116 (7.10)</td>
</tr>
</tbody>
</table>

*Region information only available for 6343 isolates.

*Number/percent of isolates with region information available (n = 6343).

*Serotypes Agona and Newport were involved in special projects; serotypes Choleraesuis and Typhimurium included the primary serotype and variants.

*Number/percent of isolates out of number of isolates in region.
Currently the VetNet program has four laboratory technicians processing PFGE Salmonella gels and one laboratory technician analyzing PFGE Salmonella gels. All five were trained and certified by CDC PulseNet.

**NARMS Campylobacter database**

The first PFGE TIFF image analysis of Campylobacter isolates for the NARMS Campylobacter database began in December of 2005. At the end of one month, the database contained 58 Campylobacter patterns, 47 C. jejuni and 11 C. coli. All isolates were cultured from chicken carcass rinsates. Antimicrobials and resistance breakpoints were: azithromycin ($\geq 2 \mu g/mL$), ciprofloxacin ($\geq 4 \mu g/mL$), chloramphenicol ($\geq 32 \mu g/mL$), clindamycin ($\geq 4 \mu g/mL$), erythromycin ($\geq 32 \mu g/mL$), gentamicin ($\geq 16 \mu g/mL$), nalidixic acid ($\geq 32 \mu g/mL$), and tetracycline ($\geq 16 \mu g/mL$). By the end of 2005, the database contained the antimicrobial resistance profiles for all 58 Campylobacter patterns. Antimicrobial resistance profiles can also be found at www.ars.usda.gov/main/site_main.htm?modecode=66-12-05-08.

For each isolate pattern, the following information, if it was known, was included in the database: species, pattern number, country where the isolate was sampled, the site of the sample (all were chicken carcass rinsate), the date the carcass rinsate was collected, the date the PFGE gel was processed and which restriction enzyme was used, the date the PFGE gel was analyzed, and the PFGE gel number and lane number where the isolate was run.

Compared to the NARMS Salmonella database, the Campylobacter database is much more diverse. Fifty-three unique Smal patterns were identified from the 58 total patterns. Only four C. jejuni patterns were represented by more than one isolate, and all 11 of the C. coli patterns were unique (Fig. 2). Currently, three laboratory technicians in the VetNet program are processing Campylobacter PFGE gels and one laboratory technician is analyzing them using PulseNet standard operating procedures. Percent of isolates was calculated from the total number of isolates in the database ($n = 58$).

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**FIG. 1.** National Antimicrobial Resistance Monitoring System (NARMS) Salmonella database top PFGE patterns. Salmonella DNA for PFGE was digested with $X_{ba}l$ and TIFF images were analyzed using PulseNet standard operating procedures. Serotype Newport isolates included those from a special project; serotypes Choleraesuis and Typhimurium included the primary serotype and variants. Percent of isolates was calculated from the total number of isolates in the database ($n = 6763$).

**FIG. 2.** National Antimicrobial Resistance Monitoring System (NARMS) Campylobacter database top PFGE patterns. Campylobacter DNA for PFGE was digested with Smal and TIFF images were analyzed using PulseNet standard operating procedures. Percent of isolates was calculated from the total number of isolates in the database ($n = 58$).
certified to run PFGE Campylobacter gels and all three technicians are certified to analyze Campylobacter PFGE gels. All laboratory personnel were trained and certified by CDC PulseNet.

CONCLUSION

Because food is often implicated during microbial foodborne outbreaks, the need for a program that would provide additional data to examine bacterial isolates cultured from animals and food is great and would add significant information to the outbreak investigation. To meet this need, the USDA-ARS established USDA VetNet (Gerner-Smidt et al., 2006). Modeled after PulseNet USA, VetNet utilizes slaughter, diagnostic, and processing isolates collected as part of the animal arm of NARMS. In addition to providing descriptive data on the extent and temporal trends of antimicrobial resistance in selected bacterial organisms from the animal population, the animal arm of NARMS also aims to facilitate identification of antimicrobial resistance in animals, provide this information in a timely fashion, use the information to promote prudent and judicious use of antimicrobials, and identify areas for increased investigation (Fedorka-Cray et al., 2002; Marano et al., 2000).

Subtyping of bacterial isolates for VetNet collected from the animal arm of NARMS provides a tremendous boost to the VetNet program due to the amount of information about the bacterial isolates in the NARMS database. In addition to antimicrobial susceptibility data, the NARMS database contains information on the source, isolation date, serotype, and region of isolation. Currently, USDA VetNet is at the initial stages of growth and development, with six technical staff members for subtyping Salmonella and Campylobacter using PFGE. Nonetheless, the program has been successful in subtyping more than 6000 Salmonella isolates since its inception. Due to the large number of Salmonella isolates collected by the animal arm of NARMS annually, Salmonella isolates submitted to NARMS beginning in fiscal year 2004 were scheduled to be processed before retrospective study of older isolates. All PFGE patterns for retail meats are submitted by FDA-CVM to PulseNet, and those profiles can be found on the FDA-CVM NARMS website at www.fda.gov/cvm/narms_pg.html#Data.

In the future, the VetNet databases will be available to additional laboratories for pattern viewing and submitting. Summary reports will also be available on the web as part of the NARMS report. This will allow a greater number of patterns to be compared to the human patterns already contained in the PulseNet database. These comparative data will be used for the surveillance and investigation of foodborne disease outbreaks. Although patterns in VetNet are named consistently with CDC protocol, pattern numbers in VetNet do not match those in PulseNet. Each organization names patterns independently in order to avoid having unassigned patterns in the database. Also, due to the source of isolates, patterns in the VetNet database may not be found in the PulseNet database. However, identification of indistinguishable PFGE patterns in both VetNet and PulseNet will prove valuable when an outbreak is in progress and may suggest possible source-related information for further investigation.

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DISCLAIMER

The 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 United States Department of Agriculture.

REFERENCES

Barrett TJ, Lior H, Green JH, et al. Laboratory investigation of a multistate food-borne outbreak of Escherichia coli

Birren BW, Hood L, Lai E. Pulsed field gel electrophoresis: studies of DNA migration made with the program-
mable, autonomously-controlled electrode electrophore-

Buchrieser C, Brosch R, Catimel B, Rocourt J. Pulsed-field gel electrophoresis applied for comparing Listeria mono-


Fedorka-Cray PJ, Englen MD, Gray JT, Hudson C, Head-
rick ML. Programs for monitoring antimicrobial resis-


Marano NN, Rossiter S, Stamey K, et al. The National Anti-

Olive DM, Bean P. Principles and applications of meth-

Olsen SJ, Hansen GR, Bartlett L, et al. An outbreak of Campylobacter jejuni infections associated with food han-


Tankson JD, Fedorka-Cray PJ, Jackson CR, Headrick M. Genetic relatedness of a rarely isolated Salmonella: Salmonella enterica serotype Niakhar from NARMS animal iso-


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