The National Antimicrobial Resistance Monitoring System: Enteric Bacteria

Introduction

This report summarizes the major findings of the National Antimicrobial Resistance Monitoring System (NARMS) for calendar year 2014, including the most important resistance findings for Salmonella and Campylobacter, Escherichia coli (E. coli) and Enterococcus.

Salmonella and Campylobacter are the leading bacterial causes of foodborne illness in the United States. The latest data (2014) from the CDC ranked Salmonella first in incidence at 15.5 cases per 100,000 inhabitants resulting over 2,100 hospitalizations and 30 deaths. Campylobacter ranked second in incidence at 13.5 cases per 100,000 resulting in 1,080 hospitalizations and 11 deaths.

E. coli and Enterococcus from cecal and retail meat samples also are tested for antimicrobial susceptibility. While these are not major food-borne disease-causing organisms, these are used as indicator organisms for testing of resistance to antimicrobials that are active against gram-negative and gram-positive bacteria, respectively.

This report presents consolidated information from the four data sources that form the NARMS system: 1. Human clinical isolates; 2. (and 3.) Food-producing animal isolates from both cecal (intestinal) isolates from slaughter animals and isolates from processing plants collected as part of Pathogen Reduction/Hazard Analysis Critical Control Point (PR/HACCP) testing; and 4. Raw retail meats.

Isolates from human laboratory-confirmed infections are tested for susceptibility to antimicrobials, and compared with bacteria derived from various stages in the food production chain. This includes cecal (intestinal) samples that are collected at slaughter from eight animal production classes - young chickens, young turkeys, dairy cows, beef cows, steers, heifers, market swine and sows, along with (PR/HACCP) isolates from the processing line recovered from chicken carcasses rinses, turkey carcass swabs, ground beef and beef trimmings, and ground or comminuted chicken and turkey products. Salmonella and Campylobacter isolates are collected monthly from four retail meat products (chicken, ground turkey, ground beef and pork chops) purchased at retail outlets in 14 states.
This report focuses on antimicrobial resistance to drug classes that are most important to human medicine (generally, first or second line treatments), multidrug resistance and specific co-resistance profiles of epidemiological importance. The details on all antimicrobial resistance findings are contained in the summary data tables and the interactive graphs.

It should be noted that due to sampling and design limitations, the temporal data comparisons for some food commodities and sampling points are more meaningful than for others. Readers are cautioned that some of the findings may be based on small differences or a small number of isolates and are encouraged to reference the full report for more details.

**What is New?**

**New Interactive Data Displays:** To accompany the NARMS Integrated Reports, the FDA has published online new interactive data displays and the bacterial isolate-level data. The enhanced interactive data displays allow users to explore trends in resistance to antimicrobial agents by host, sample type, bacterial species, and serotype. Users are also able to visualize the prevalence of *Salmonella* resistance genes by source. The isolate level database, which was originally published in August 2015, contains the entire collection of NARMS enteric bacterial isolates tested and shows data for *Salmonella*, *Campylobacter*, *E. coli*, and *Enterococcus*. For this report, the database has been updated with information on *Salmonella* resistance genes and gene mutations associated with resistance.

**Whole Genome Sequence (WGS) Data:** The advent of WGS is a major advance for integrated surveillance of antimicrobial resistance. By providing the complete DNA sequence of a bacterial genome, this technology allows detailed genetic comparison of strains from humans with those from other sources. In addition, studies show that the presence of known resistance determinants is highly correlated with clinical resistance (see TEXT BOX 4).

*Salmonella* WGS data from all three component of the NARMS program accompany the 2014 NARMS Integrated Report. For *Salmonella* from human infections, all isolates exhibiting resistance to any antimicrobial drug were subject to WGS. For *Salmonella*-positive retail meat samples and cecal samples, WGS was performed on every isolate recovered in 2014. In addition, the WGS has been determined for historical *Salmonella* (over 4,500 isolates) recovered from retail meat sources since testing began in 2002. The genomes have been uploaded to the public database at the National Center for Biotechnology Information (NCBI). As these data accumulate, NARMS reports will evolve to incorporate temporal changes in the resistome along with the susceptibility information.

**New Breakpoint for Streptomycin:** Because the Clinical and Laboratory Standards Institute (CLSI) has not defined resistance breakpoints in streptomycin in *Salmonella*, NARMS has always
used its own minimum inhibitory concentration (MIC) values. In 2014, the testing range for streptomycin was expanded in order to determine the full range of MIC values. These data, along with WGS information, were used to lower the resistance breakpoint from ≥ 64 µg/ml to ≥ 32 µg/ml. Because the historical testing range only included two streptomycin concentrations of 32 µg/ml and 64 µg/ml, this new breakpoint will be applied to all testing beginning with the 2014 isolates.

Pathogenic Bacteria

Nontyphoidal *Salmonella*

Nontyphoidal *Salmonella* (i.e., serotypes other than Typhi, Paratyphi A, Paratyphi B, and Paratyphi C) usually cause diarrhea, fever and abdominal cramps. Some infections spread to the blood and can be life threatening. Nontyphoidal *Salmonella* infections cause an estimated 1.2 million illnesses, 23,000 hospitalizations, and 450 deaths each year in the United States (1). Direct medical costs are estimated to be $3.6 billion annually (2).

Physicians rely on antimicrobial drugs such as ceftriaxone and ciprofloxacin for treating patients with severe *Salmonella* infection. Therefore, preventing resistance to these antimicrobials is important. It is estimated that approximately 100,000 drug-resistant nontyphoidal *Salmonella* infections and 40 deaths occur annually in the United States (3).

Prevalence of Nontyphoidal *Salmonella*

In 2014, a total of 5,043 nontyphoidal *Salmonella* isolates were tested by NARMS, 2,127 from humans, 262 from retail meats, 1,579 from PR/HACCP samples and 1,075 from cecal samples.

For retail meat testing in 2014, *Salmonella* recovery continued to decline in poultry sources to the lowest levels in 20 years of NARMS testing, reaching a prevalence of 9.1% in chicken and 5.5% in ground turkey, while remaining below 1.5% in beef (0.8%) and pork (1.3%) (Figure 1).
Cecal samples were included in NARMS for the first time in 2013. This new design allowed for a randomized and nationally representative sampling of U.S. national food animal production. In 2014, the prevalence of *Salmonella* did not change substantially. *Salmonella* was isolated from 18% of chickens, 17% of turkeys, 6% of beef cows, 20% of dairy cows, 38% of market swine and 58% of sows (Figure 2).
Antimicrobial Resistance in Nontyphoidal *Salmonella*

In 2014, 82% of *Salmonella* isolated from humans had no resistance to any of the antimicrobial drugs tested. Among bacteria from retail meats and PR/HACCP samples, isolates from turkey sources were historically most frequently resistant to at least one antimicrobial, and those from bovine sources were least frequently resistant (Figure 3). Analysis of PR/HACCP and cecal data showed some differences in antimicrobial resistance between these two sample types for turkey and bovine sources (see TEXT BOX 1).
TEXT BOX 1

Comparison of Susceptibility Profiles in *Salmonella* Isolates collected in the NARMS PR/HACCP and Cecal Sampling Programs, 2014

In 2013, the USDA Food Safety and Inspection Service (FSIS) implemented a sampling program to culture cecal (intestinal) contents of cattle, poultry, and swine. This effort was launched to enhance representativeness of the animal monitoring component. Because cecal bacteria are collected prior to slaughter and processing interventions, they better reflect animal bacterial populations from the farm. This program complements FSIS's existing Pathogen Reduction/Hazard Analysis and Critical Control Point (PR/HACCP) sampling program which collects samples at the end of processing. We sought to compare susceptibility phenotypes of these two sample sets to understand the influence of processing on resistance.

Although NARMS cecal and PR/HACCP sampling complement each other, comparing the results between the two programs is complicated by differences in sampling design. First, cecal sampling includes swine (in addition to cattle and poultry) while PR/HACCP sampling does not. Second, cecal samples are collected from federally-regulated establishments with frequency determined by slaughter volume and thus, are more nationally representative than PR/HACCP samples, which historically have been subject to regulatory risk-based criteria and not designed to estimate *Salmonella* prevalence. In addition, as a matrix cecal samples are more uniform within and across commodities, whereas PR/HACCP samples vary depending on commodity and include ground product (beef, chicken, and turkey), carcass rinses (chicken), and carcass swabs (turkey). Despite these differences, valuable information regarding AMR persistence and variation from pre-harvest through slaughter and processing can be obtained by comparing cecal and PR/HACCP sampling results.

Multiple drug resistance, *Salmonella* isolates, 2014

> 3 antimicrobial classes

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1 For cattle, ‘cecal’ includes beef cows, steers and heifers

Continued on page 7
Differences in AMR in *Salmonella* isolates were observed between and within commodities sampled via cecal and PR/HACCP. Cattle PR/HACCP isolates exhibited higher percent resistance to one or more antimicrobial drugs (27.8% PR/HACCP vs 18.0% cecal; OR=1.8, 95% CI: 1.2-2.7) and a higher proportion of multidrug resistance (resistance to 3 or more antimicrobial classes MDR) (16.6% PR/HACCP vs 10.1% cecal; OR=1.8, 95% CI:1.0-13.1) than dairy cow cecal isolates. In contrast, among chicken samples, cecal isolates showed greater resistance to sulfisoxazole (9.6% PR/HACCP vs 29.1% cecal; OR=0.7, 95% CI: 0.2-0.4) than those sourced via PR/HACCP sampling, a higher proportion of MDR (8.3% PR/HACCP vs 14.6% cecal; OR=0.5, 95% CI: 0.3-1.0) and a higher proportion of streptomycin resistance among PR/HACCP isolates (41.8% PR/HACCP vs 30.1% cecal; OR=1.7, 95% CI: 1.1-2.6). Cecal and PR/HACCP isolates from turkeys presented similar resistance to most antimicrobial drugs.

On a positive note, resistance to azithromycin and ciprofloxacin, critically important antimicrobials, was either absent or low in both cecal and PR/HACCP isolates (n ≤ 2 isolates in each commodity). In isolates sourced from chicken and turkey, ceftriaxone resistance was similar in PR/HACCP and cecal sampling. Cattle PR/HACCP and dairy cecal results also were similar. The largest difference observed in ceftriaxone resistance was between isolates sourced from beef samples via PR/HACCP (7.5%) and cecal (1.0%) (OR=8.4, 95% CI:1.1-347.5 Fisher CI). Note: OR is calculated as a ratio of a/b divided by c/d. PR/HACCP values are designated as ‘a’ and cecal as ‘b’. As ‘c’ and ‘d’ are dependent on ‘a’ and ‘b’, this pattern of designation results in an OR of less than 1 when the cecal values are higher than PR/HACCP.

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For cattle, ‘cecal’ includes beef cows, steers and heifers

Summary:

Specific AMR differences seen in PR/HACCP may be influenced by in-plant processing factors, while resistance among cecal isolates may reflect on-farm antimicrobial usage or other animal exposures occurring on farm or during transit to slaughter. There are ongoing efforts to explore appropriate methods to analyze changes in antimicrobial resistance from farm to retail.
Quinolone Resistance

In the United States, fluoroquinolones (e.g., ciprofloxacin) are critically important for treating severe Salmonella infections in adults. Other fluoroquinolones (e.g., enrofloxacin) are approved for the treatment and control of certain respiratory infections in swine and cattle, and for the control of diarrhea associated with E. coli in weaned pigs. Since the FDA withdrawal of enrofloxacin for poultry in 2005, fluoroquinolones are no longer approved for use in chickens and turkeys. The extra-label use of fluoroquinolones in food-producing animals has been prohibited since 1997.

Data in 2014 continue to demonstrate that ciprofloxacin resistance is uncommon in Salmonella isolates from sources monitored in NARMS (Figure 4). The percentage of Salmonella isolates that fall in the category of “non-susceptible” to ciprofloxacin (those with both resistant and intermediate MIC values) has been below 10% since 1996 when the NARMS program began. Since the FDA rescinded the poultry approval for fluoroquinolones in 2005, nalidixic acid resistance in Salmonella has not exceeded 3% in isolates from turkey sources and 0.7% in isolates from chicken sources.

Figure 4. Nalidixic Acid Resistance in Nontyphoidal Salmonella
Traditionally, nalidixic acid resistance has been used to indicate the first of two mutations required for full resistance to ciprofloxacin. Recent data suggest that nalidixic acid and ciprofloxacin resistance trends are diverging in human nontyphoidal *Salmonella*, possibly due to increased presence of plasmid mediated quinolone resistance genes (see 2014 NARMS Annual Human Isolates Report), which may confer resistance to ciprofloxacin but not nalidixic acid. The presence of such plasmid-associated resistance genes is of concern due to the potential for transmission, either alone or together with other resistance genes, to susceptible strains of *Salmonella*. In 2014, FDA identified the first instance of ciprofloxacin-resistant *Salmonella* in a single isolate from a retail pork sample which carried the *qnrS* gene. This appears to be the first report of *qnr* genes present in retail meat *Salmonella* isolated in the United States.

Although nalidixic acid resistance remains low overall, it has steadily increased in *Salmonella* isolates from human clinical cases from 0.4% in 1996 to a high of 3.5% in 2014 (Figure 4). This trend is driven mainly by an increase in nalidixic acid resistance among serotype Enteritidis, the most common serotype in human illness. Studies by the CDC indicated that many of these infections were likely acquired during foreign travel (4). In addition, nalidixic acid resistance has increased in cattle PR/HACCP *Salmonella* isolates from 0% in 1997 to 2.3% in 2014, and decreased among PR/HACCP turkey isolates from 5.3% in 2002 to 0.7% in 2014.

**Cephalosporin Resistance**

Extended-spectrum cephalosporins such as ceftriaxone are critically important drugs for treating severe *Salmonella* infections, especially in children. A related cephalosporin, ceftiofur, is approved for therapeutic use in food-producing animals (Animal Drugs @ FDA). Resistance to one compound results in cross-resistance to the other.

Ceftriaxone resistance increased in *Salmonella* recovered from retail turkey and chicken between 2002 and 2010 (Figure 5). The increases were often present as part of a multidrug resistance profile (see below). Given the critical importance of this drug class, the FDA used these findings and other data to prohibit certain unapproved uses of cephalosporin drugs in cattle, swine, chickens, and turkeys. The cephalosporin order of prohibition was announced in 2009 and went into effect in April 2012.

Among human isolates, the proportion of *Salmonella* showing ceftriaxone resistance declined from 3.4% in 2009 to 2.4% in 2014. This was paralleled by a decline in resistance from 38% to 18% in retail chicken isolates, and from 12.9% to 6% in chicken PR/HACCP isolates during this time frame (Figure 5). Among retail ground turkey isolates, resistance showed a continued decline to 7 % after peaking at 22% in 2011 (Figure 5).
Although resistance to ceftriaxone appears to be declining recently among nontyphoidal *Salmonella* (Figure 5 and Figure 6), its prevalence varies by serotype and source. *Salmonella* serotypes Heidelberg, Typhimurium and Kentucky are consistently among the most common serotypes from poultry sources.

The proportion of human *Salmonella* Heidelberg isolates with ceftriaxone resistant declined in 2014 to 8.5% from a peak of 24% in 2010 and from 15% in 2013. In Heidelberg isolates from retail chicken, ceftriaxone resistance was 0% in 2013, after peaking at 32% (14/44) in 2009, but ticked up to 12.5% (3/24) in 2014. Among retail ground turkey isolates of Heidelberg, ceftriaxone resistance was not detected in 2014, after peaking at 36% (10/28) in 2011.

Among human isolates of serotype Typhimurium, ceftriaxone resistance has remained <7% since testing began in 1996. In retail chicken isolates, however, resistance has ranged between 33% and 64% since testing began in 2002; in 2014, 47% (18/38) of the isolates were resistant.

*Salmonella* serotype Kentucky is a common serotype from poultry sources that often exhibits ceftriaxone resistance, ranging in prevalence from 12% of retail chicken to 68% of chicken
HACCP isolates. While Kentucky is not a common serotype associated with human infections, its apparent role as a reservoir of resistance to extended-spectrum cephalosporins is notable. Salmonella serotype Newport is common in cattle sources, and ceftriaxone resistance is highest in serotype Newport, where ceftriaxone resistance was found in 53% (9/17) of isolates from cattle PR/HACCP strains. Ceftriaxone resistance in *Salmonella* isolates recovered from cattle PR/HACCP sampling (Figure 6) declined to 7.5% in 2014, the lowest level since 1999.

![Figure 6. Ceftriaxone Resistance in Nontyphoidal *Salmonella* from Cattle, Swine and Humans](image)

Although it is early, it appears that the use restrictions are having a benefit. The decline in ceftriaxone resistance following FDA’s targeted prohibitions on extralabel cephalosporin use is similar to what others have observed following reductions in the use of ceftiofur in animal production (5, 6), implying that the intervention is having the intended effect on some bacteria.

One exception to the declining ceftriaxone resistance is serotype Dublin. In previous years, the proportion of isolates demonstrating ceftriaxone resistance has increased in serotype Dublin isolates from cattle PR/HACCP sources although a drop was observed in 2014. While Dublin is not a common cause of human infection, it is more likely to result in bloodstream infections than other serotypes. It is also a challenge in veterinary medicine as a common cause of
neonatal calf diarrhea. Ceftriaxone resistance among S. Dublin did decrease in 2014 but remained relatively high at 60% (6/10) of human isolates and 29% (9/31) for cattle PR/HACCP isolates (see TEXT BOX 2).

Historically, ceftriaxone resistance in U.S. Salmonella has been mediated by the \textit{bla\textsubscript{CMY-2}} gene. WGS data have revealed that a new family of ceftriaxone resistance genes, designated \textit{bla\textsubscript{CTX-M}}, are appearing in the U.S. This family of genes is common in other countries. In NARMS food and animal isolates, evidence suggests these genes are becoming more common in the U.S. (see TEXT BOX 3).

More information on ceftriaxone resistance in other serotypes is presented in the interactive data displays and in the complete data tables appended to this report.

**TEXT BOX 2**

**Antimicrobial Resistance in \textit{Salmonella} Dublin**

\textit{Salmonella} Dublin is a host-adapted serotype causing enteric and systemic disease in cattle. It is among the most common \textit{Salmonella} serotypes isolated from PR/HACCP cattle and retail ground beef sampling. Among \textit{Salmonella} isolates tested by NARMS in 2014, 9% (31/344) from PR/HACCP cattle and 23% (3/13) from retail ground beef were serotype Dublin. While Dublin is a relatively rare serotype in humans infections, it tends to cause more severe illness than other nontyphoidal \textit{Salmonella}.\textsuperscript{1,2} A 1996-2006 FoodNet study found that among nontyphoidal \textit{Salmonella} serotypes with more than 50 isolates, \textit{Salmonella} Dublin had the highest proportion of invasive infections (64%), hospitalizations (67%), and deaths (3%).\textsuperscript{2} Antimicrobial treatment is critical for invasive \textit{Salmonella} infections and ceftriaxone (for children and adults) and ciprofloxacin (for adults) are primary treatment options.\textsuperscript{3}

Over the past 13 years, ceftriaxone resistance among NARMS S. Dublin isolates from humans and PR/HACCP cattle has generally increased, reaching a peak in 2013 of 92% (11/12) and 86% (18/21), respectively. Ceftriaxone resistance among S. Dublin decreased in 2014, but remained relatively high at 60% (6/10) for humans and 29% (9/31) for cattle, accounting for 12% (6/51) and 35% (9/26) of all ceftriaxone-resistant \textit{Salmonella} isolates from those sources, respectively. Sampling of retail ground beef isolates since 2002 has identified 21 S. Dublin isolates, of which 15 (71%) were ceftriaxone resistant. Ceftriaxone-resistant S. Dublin isolates were commonly resistant to at least ampicillin, chloramphenicol, streptomycin, sulfonamides, tetracycline, and amoxicillin-clavulanic acid (ACSSuT\textsubscript{Au}C\textsubscript{x}) among humans (35/38, 92%), cattle (126/178, 71%), and retail ground beef (13/15, 87%) for all years.

\textit{S.} Dublin isolates displaying decreased susceptibility to ciprofloxacin (DSC, either intermediate or resistant: MIC \textgeq 0.12 \textmu g/mL) also have emerged. Since 2004, 9% (7/78) of Dublin isolates from humans and 12% (42/337) from cattle have displayed DSC. Among Dublin isolates with DSC, 57% (4/7) from humans and 40% (18/42) from cattle also were ceftriaxone-resistant. Three of 15 (20%) S. Dublin isolates from retail ground beef since 2009 have displayed DSC an were all ceftriaxone-resistant. The increase in ceftriaxone resistance and DSC in recent years among Dublin, a serotype that is highly invasive in humans, is a significant public health concern as treatment options for these infections may be limited.

Continued on page 13
Figure 1. Percentage of *Salmonella* serotype Dublin isolates from humans and cattle with resistance to ceftriaxone and decreased susceptibility to ciprofloxacin, 1996–2014

A. Resistance to ceftriaxone

B. Decreased susceptibility to ciprofloxacin (DSC)

Table 1. Number of *Salmonella* serotype Dublin isolates from humans and cattle sources* with resistance to ceftriaxone and decreased susceptibility to ciprofloxacin (DSC)†, 1996–2014

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<td>Ceftriaxone and DSC†</td>
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</tbody>
</table>

* Since cecal sampling began in 2013, 2 S. Dublin isolates have been tested: 1 from beef cattle in 2013 was resistant to ceftriaxone but ciprofloxacin susceptible; 1 from dairy cow sampling in 2014 was susceptible to both ceftriaxone and ciprofloxacin (data not shown)
† Decreased susceptibility to ciprofloxacin (DSC): includes isolates with MICs categorized as intermediate or resistant for ciprofloxacin (MIC ≥0.12 µg/mL)

References:
Multidrug Resistance

Multidrug resistance (MDR) is defined as resistance to 3 or more classes of antimicrobials. This categorization makes no distinction among the classes based on their importance to human medicine. Evidence shows, however, that patients with MDR Salmonella infections may have more severe clinical disease and a greater likelihood of hospitalization (7, 8).

MDR among human Salmonella isolates declined from 17% in 1996 to 9.6% in 2008, and has remained stable since, appearing in 9.3% of human isolates in 2014 (Figure 7).

In 2014 PR/HACCP isolates, MDR was more common in turkey (41%) Salmonella isolates than those from chicken (8.3%) or cattle (17%) sources. It has increased from 26.7% to 41% in turkey PR/HACCP isolates over the past 10 years (Figure 7). In 2014, MDR was high in isolates recovered from retail poultry meats, ranging from 20% to 36%.
In the NARMS 2012-2013 report, the emergence of MDR in *Salmonella* serotype Dublin was highlighted. While the incidence of human infections is relatively low, it is among the top 4 serotypes isolated from cattle-HACCP and retail ground beef. The few serotype Dublin isolates tested annually have high levels of MDR and ceftriaxone resistance, and some also exhibit decreased susceptibility to ciprofloxacin.

**TEXT BOX 3**

**Surveillance for ESBL and Carbapenemase-Producing Bacteria in Food and Food Animals**

The β-lactams are a class of antibiotic drugs that inhibit cell wall synthesis in the target microorganism. One way bacteria can develop resistance to these drugs is by producing β-lactamase enzymes that bind to and inactivate the drug. There are several major classes of β-lactamase enzymes, including penicillins, AmpC-type cephalosporinases, extended spectrum beta-lactamases (ESBL), and carbapenemases. While the first two classes of enzymes are commonly found in β-lactam-resistant foodborne bacteria in the United States, the second two classes are not as common.

ESBLs are enzymes that confer resistance to most of the β-lactam antibiotics, including penicillins, expanded-spectrum cephalosporins, and monobactams, but not cephemycins or carbapenems. Carbapenemases are enzymes that confer resistance to all β-lactams, including carbapenems. Infections with ESBL and carbapenemase-producing organisms are particularly concerning because they are resistant not only to most of the β-lactam antibiotics but they are typically resistant to additional classes of antibiotics, leaving few treatment options often resulting in poor clinical outcomes. Of the ESBL enzymes, the CTX-M family is the most widely disseminated in the world, particularly in areas of Europe and Asia. Other common ESBL types include SHV and TEM.

During 2012-2014, extended-spectrum cephalosporin-resistant *Salmonella* from all sources and *E. coli* from retail sources were screened for susceptibility patterns that may indicate the presence of ESBLs and carbapenemases. Isolates were selected based on CLSI criteria for presumptive ESBL-positive bacteria, and subjected to whole genome sequencing to identify putative ESBL- and carbapenemase-encoding genes. Tables 1 and 2 summarize the findings from 2012-2014.

Most of the potential ESBL producing organisms contained genes belonging to the CTX-M family of enzymes, and 4 isolates from 2014 were found to harbor variants of the SHV family. Although ESBL genes are not frequently reported in isolates of animal origin in the U.S., ESBL-producing *E. coli* and *Salmonella* have been found in livestock (1-5) and ESBL producing *E. coli* have been recovered from retail foods (6). However, this was the first time we observed ESBL producing *Salmonella* in retail meat.

Carbapenem resistance remains a rare occurrence among NARMS isolates. Carbapenem drugs are not approved for use in food animals. We have previously reported two cases of infection by *Salmonella* that contained the carbapenemase genes [blaKPC (7) in 1998 and blaNDM 1 in 2011 (8), we have not found any additional carbapenemase-producing *Salmonella* or *E. coli* in human, retail food, or cecal samples collected for routine NARMS surveillance. There are currently no other reports of carbapenemases in foods or food animals.

Continued on page 16
Table 1. Number of *Salmonella* Containing ESBLs*
(Total number of *Salmonella* in bold)

<table>
<thead>
<tr>
<th></th>
<th>2012</th>
<th>2013</th>
<th>2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humans</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>bla</em>CTX-M-14 (1)</td>
<td><em>bla</em>CTX-M-65 (2)</td>
<td><em>bla</em>CTX-M-65 (2)</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Retail</td>
<td></td>
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</tr>
<tr>
<td>Chickens</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Ground Turkey</td>
<td><em>bla</em>CTX-M-1 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cecal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef Cows</td>
<td>NA</td>
<td><em>bla</em>SHV-12 (1)</td>
<td></td>
</tr>
<tr>
<td>Market Swine</td>
<td>NA</td>
<td><em>bla</em>SHV-12 (1)</td>
<td></td>
</tr>
<tr>
<td>PR/HACCP</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Turkey**</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>5</td>
<td>11</td>
</tr>
</tbody>
</table>

*Only sources containing ESBL positive bacteria are shown. Grey boxes indicate no ESBL positive isolates found.

**ESBL positive *Salmonella* isolates were detected in 2014 FSIS sampling of ground turkey (2/299 *Salmonella* isolates) and sampling of mechanically separated turkey (2/70 MST *Salmonella* isolates) and mechanically separated chicken (8/1208 MSC *Salmonella* isolates). *Salmonella* isolates from mechanically separated poultry samples are not included in poultry PR/HACCP totals in this report. Depending on the time and source of the data retrieval the denominators may show a slight change. All 4 of the turkey ESBL *Salmonella* isolates were unidentified; in MSC 6 isolates were *bla*CTX-M-65, 1 isolate was *bla*CTX-M-1 and 1 isolate was unidentified.

NA: Source not screened

Table 2. Number of *E. coli* Containing ESBLs*
(Total number of *E. coli* in bold)

<table>
<thead>
<tr>
<th></th>
<th>2012</th>
<th>2013</th>
<th>2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retail</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chickens</td>
<td><em>bla</em>CTX-M-1 (2)</td>
<td><em>bla</em>CTX-M-1 (1)</td>
<td></td>
</tr>
<tr>
<td>Ground Turkey</td>
<td><em>bla</em>CTX-M-15 (1)</td>
<td><em>bla</em>CTX-M-1 (1)</td>
<td></td>
</tr>
<tr>
<td>Ground Beef</td>
<td><em>bla</em>CTX-M-15 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pork Chops</td>
<td><em>bla</em>CTX-M-14 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

*Only sources containing ESBL positive bacteria are shown. Grey boxes indicate no ESBL positive isolates found.

References
An important MDR pattern in *Salmonella* is combined resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (ACSSuT). ACSSuT resistance is commonly found in *Salmonella* Typhimurium DT104 strains and has steadily declined in the U.S. Among all serotypes, the percentage of human isolates resistant to at least ACSSuT continues a steady decline to 3.1%, the lowest since testing began in 1996 (Figure 8). This MDR pattern is most common in cattle PR/HACCP isolates, where it was detected in 7% of the isolates in 2014, down from a peak of 20% in 2005. In poultry isolates of *Salmonella*, ACSSuT resistance has been consistently <5%.

![Figure 8. ACSSuT Resistant Nontyphoidal Salmonella](image)

In human *Salmonella* Typhimurium, the percentage of isolates resistant to at least ACSSuT has declined since 1996 to 14.5% in 2014 (Figure 9). Similarly, ACSSuT resistance in cattle PR/HACCP isolates of Typhimurium declined sharply from 67% in 2009 to 7.1% in 2014, the lowest level since testing began in 1997. The levels of ACSSuT resistance in Typhimurium from dairy cecal samples remained high (60%; 6/10) but this pattern was not detected in beef cow cecal isolates in 2014. Declining MDR in Typhimurium is the main driver behind overall declining MDR *Salmonella* in human isolates (9).
The ACSSuT profile can occur with resistance to additional beta-lactam drugs, such as ceftriaxone and amoxicillin-clavulanic acid. This phenotype (abbreviated as MDR-AmpC or ACSSuTAuCx) has been detected in *Salmonella* isolates from all NARMS sample sources over the years. The ACSSuTAuCx resistance profile is most common in cattle isolates, where it was found in 6.1% of cattle PR/HACCP strains and 30.8% of retail ground beef strains in 2014 with no apparent trends.

**Extremely Drug Resistant (XDR) Salmonella**

For the purposes of this report, XDR is defined as resistance to all, or all but one, of the nine antimicrobial classes tested in NARMS. In 2014, 2 XDR *Salmonella* strains were identified in NARMS human isolate testing: 1 serotype Typhimurium and 1 serotype Enteritidis. These isolates were resistant to all classes except macrolides.

Among cattle sources, 4 PR/HACCP ground beef and 1 retail ground beef isolate showed resistance to all 9 classes except macrolides; and 1 cecal isolate from a dairy cow was resistant to 8 classes including macrolides, but was susceptible to quinolones. The ground beef isolates
were serotype Reading (n=2) and Dublin (n=3). The dairy cow isolate was serotype Montevideo. There also was one Agona isolate from market swine cecal sampling and 2 PR/HACCP turkey sampling isolates (serotypes Albert and Heidelberg) that were resistant to all drug classes except quinolones. No chicken isolates were XDR.

**TEXT BOX 4**

**The use of whole genome sequencing analysis for detecting antimicrobial resistance**

As in other fields of biology, whole genome sequencing (WGS) technology is transforming microbiology and infectious disease surveillance. WGS offers a single cost-effective method to identify and characterize microbes, including the ability to identify antimicrobial resistance genes. We evaluated correlations between resistance phenotypes and the presence of known resistance genes in a collection of 114 *Campylobacter*, 76 *E. coli* and 640 *Salmonella* isolates from human, animal and retail meat isolates from NARMS. Known antimicrobial resistance genes were downloaded from the GenBank, and resistance genotypes were determined using assembled WGS sequences through Basic Local Alignment Search Tool (BLAST) analysis with the cutoff set at 50% sequence length and 85% amino acid identity.

Among 114 *Campylobacter* isolates, eighteen resistance genes, and mutations in two house-keeping genes (gyrA and 23S rRNA) were identified. There was a high degree of correlation between phenotypic resistance to a given drug and the presence of one or more corresponding resistance genes. Phenotype and genotype correlated in 100% of isolates showing resistance to tetracycline, ciprofloxacin/nalidixic acid, and erythromycin; and ranged from 95.4% to 98.7% for gentamicin, azithromycin, clindamycin, and telithromycin. All isolates were susceptible to florfenicol, and no genes associated with florfenicol resistance were detected.

In *E. coli*, over 30 resistance genes and a number of resistance mutations were identified among 76 isolates. Resistance genotypes correlated with 97.8% specificity and 99.6% sensitivity to the identified phenotypes. A majority of discordant results were attributable to the aminoglycoside streptomycin, whereas there was a perfect genotypic-phenotypic correlation for most antibiotic classes such as tetracyclines, quinolones, and phenicols.

Among 640 *Salmonella*, a total of 65 unique resistance genes, plus mutations in two housekeeping genes, were identified. Isolates from human sources had greater resistance gene diversity (n=59 distinct genes) than isolates recovered from retail meat (n=36 genes). Overall, resistance genotypes and phenotypes correlated in 99.0% of cases. Correlations approached 100% for most classes of antibiotics, but were lower for aminoglycosides and beta-lactams. This study also revealed the first isolates of *Salmonella* in the U.S.

Overall, resistance genotypes and phenotypes correlated in 99.0% of tests for all three organisms, ranging from 86%-100% for all 19 drugs tested. These results show that WGS can accurately predict resistance phenotypes for most drugs for which genetic resistance mechanisms have been defined. By providing details on the alleles present in strains from different sources, WGS provides a powerful tool for antimicrobial resistance surveillance.

References:

**Campylobacter**

*Campylobacter* is estimated to cause over 1.3 million illnesses and 120 deaths in the United States each year (1). Most people who become ill from *Campylobacter* develop diarrhea, abdominal pain and fever. Approximately 90% of human *Campylobacter* infections are caused by *Campylobacter jejuni* and nearly 10% by *Campylobacter coli*. Case-control studies have shown poultry to be a major food source for these infections.

**Prevalence of Campylobacter**

A total of 4,122 *Campylobacter* isolates were tested in 2014. The distribution of *Campylobacter* species by source is shown in Figure 10.

![Figure 10. Campylobacter species distribution by source, 2014](image)

For retail meat testing in 2014, *Campylobacter* was isolated from 33% of retail chicken samples (Figure 11). The prevalence of *Campylobacter* cultured from retail chicken meat samples has declined steadily, down 37% (from 52% to 33%) since 2003. For cecal samples, *Campylobacter* was isolated from 12% of chickens, 6.1% of turkeys, 42% of beef cows, 42% of dairy cows, 25% of market swine and 28.3% of sows (Figure 12).
Figure 11. Retail Meat Samples with Positive Cultures for *Campylobacter*, 2002-2014

![Chart showing the percentage of positive cultures for Campylobacter in retail meat samples from 2002 to 2014. The chart displays two lines: one for Retail Chicken and another for Retail Ground Turkey. The percentage of positive cultures varies over the years, with peaks and troughs. The chart indicates a trend of decreasing positive cultures in Retail Ground Turkey samples.]

Figure 12. Prevalence of *Campylobacter* in Animal Cecal Samples, 2013-2014

![Bar chart showing the prevalence of Campylobacter in animal cecal samples from 2013 to 2014. The chart includes data for chickens, turkeys, cattle, dairy, market swine, and sows, with a focus on the year 2014. The chart displays the number of Campylobacter samples tested and the corresponding number of positive samples. The data shows variations in the prevalence rates across different animal groups and years.]

- No. of *Campylobacter* samples tested
- Percent Positive
- Year
- Chickens
- Turkeys
- Cattle
- Dairy
- Market Swine
- Sows

Data for 2014:
- Chickens: 754 samples, 16 positive
- turkeys: 660 samples, 16 positive
- Cattle: 652 samples, 199 positive
- Dairy: 565 samples, 160 positive
- Market Swine: 730 samples, 565 positive
- Sows: 534 samples, 565 positive
Antimicrobial Resistance in *Campylobacter*

**Macrolide Resistance**

Macrolides are first line therapies for the treatment of severe campylobacteriosis in humans (10). Macrolides also are authorized for use in all major classes of food producing animals (11).

*C. jejuni* from both human and chicken sources have exhibited erythromycin resistance rates of less than 4% since NARMS testing began (Figure 13). In 2014, erythromycin resistance in *C. jejuni* was below 2% in isolates from all sources except for market hog cecal isolates, where resistance was present in 2 of 9 isolates (22%). Among *C. jejuni* cecal isolates from cattle, erythromycin resistance was very low in both beef cows (0.3%) and dairy cows (0.3%) isolates (Figure 14).

![Figure 13. Erythromycin Resistance in *C. jejuni* from Poultry and Humans](image)

Historically, *C. coli* isolates are more commonly resistant to erythromycin and other agents than are strains of *C. jejuni*. The 2012-2013 NARMS report noted that erythromycin resistance had more than doubled among human, retail chicken and PR/HACCP chicken isolates of *C. coli* between 2011 and 2013. As a result, molecular testing was performed on human isolates to determine if the rapid increase in resistance was due to a recently-discovered transmissible macrolide resistance gene (see TEXT BOX 5). The macrolide resistant isolates from NARMS were found to harbor only the standard chromosomal mutations in the 23S ribosomal RNA genes.
In 2014, erythromycin resistance in human and retail chicken isolates of *C. coli* remained higher than 2011 levels (10.3% vs. 2.7% and 11.4% vs. 5.2%, respectively) (Figure 15), whereas resistance among PR/HACCP chicken isolates had dropped back to 2011 levels (3.8% vs. 3.4%). Among the 2014 cecal samples collected at slaughter, macrolide resistance was found in 21% of *C. coli* isolates from sows, 40% from market swine, 11% from chickens, 6.7% from turkeys, 3.3% of beef cows and 9.1% of dairy cows (Figure 14).

**Figure 14. Erythromycin Resistance in *Campylobacter* from Animal Ceca, 2013-2014**

**Figure 15. Erythromycin Resistance in *C. coli* from Poultry and Humans**
TEXT BOX 5

Genetic Characterization of Increased Macrolide Resistance in *Campylobacter coli*

NARMS data showed an increase in macrolide-resistant *C. coli* in recent years, more than doubling between 2011 and 2012 in human and poultry strains, and nearly doubled again in human isolates from 2012 (9%) to 2013 (16.9%), before and declining in 2014 (Figure 1).

To determine whether this increase was due to monoclonal or polyclonal expansion, or to the rapid dissemination of a new transmissible element, we performed whole genome sequencing (WGS) on 12 macrolide-resistant isolates from 2013. We were particularly interested to ascertain whether the plasmid-borne *erm(B)* gene, which has been found in *Campylobacter* from China, might be driving this change. This mechanism could change the dynamics of macrolide resistant, multidrug resistant *Campylobacter* in the U.S.

While several antibiotic resistance genes conferring resistance to aminoglycosides, β-lactams, and tetracycline were identified, only structural mutations in the 23S ribosomal gene (including A2074G) commonly associated with macrolide resistance were found. Comparative genome analysis indicated that the 12 *C. coli* were not closely related, suggesting that macrolide resistance is not emerging because of clonal expansion of resistant strains.

Figure 1. Macrolide resistance in human and poultry isolates of *Campylobacter coli*
**Fluoroquinolone resistance**

The fluoroquinolones are an alternative therapy for treating campylobacteriosis in adults (Allos and Blaser, 2010). Fluoroquinolones have not been used in chickens and turkeys since 2005. Currently, there are FDA approvals for fluoroquinolones in swine and certain classes of cattle, and off-label uses are prohibited (11).

Ciprofloxacin resistance among *C. jejuni* from humans in 2014 (27%) was the highest it has been since testing began in 1998, up from 2013 levels (22%). Between 2013 and 2014, there was also an increase in resistance among retail chicken (11% to 15%) and PR/HACCP chicken isolates (24% to 28%) (Figure 16).

In cattle, ciprofloxacin resistance was detected in *C. jejuni* recovered from dairy cows (8.4%) and beef cow cecal isolates (16%) (Figure 18). Isolate numbers were too low (fewer than 15 isolates) to evaluate the turkey samples and the chicken and swine cecal samples. 

![Figure 16. Ciprofloxacin Resistance in C. jejuni from Poultry and Humans](image)
Fluoroquinolone resistance trends for C. coli are shown in Figure 17. As seen in the previous three years, ciprofloxacin resistance in Campylobacter from humans was high (36%) (Figure 17). Compared to 2013, ciprofloxacin resistance in 2014 either stayed the same or decreased in C. coli isolated from poultry sources. Increases were observed among C. coli isolated from cattle and swine ceca, however, where resistance was high among beef (62%) and dairy (47%) cecal isolates (Figure 18).
Indicator Bacteria

*Escherichia coli*

*Escherichia coli* are monitored as an indicator organism for emerging resistance patterns and specific resistance genes that could be transferred to other pathogenic gram-negative bacteria (e.g., *Salmonella*). *E. coli* are tested for susceptibility to the same antimicrobials used in *Salmonella* testing. This report includes data on generic *E. coli* isolated from all four retail meat commodities and from food animal cecal samples. Ongoing antimicrobial resistance surveillance is not conducted for indicator organisms from healthy human populations.
Prevalence of *E. coli*

*E. coli* is an indicator of fecal contamination and is commonly present in raw retail meat products. In 2014, a total of 2,025 *E. coli* isolates from retail meats and cecal samples were tested by NARMS. The prevalence of *E. coli* from the tested retail meat sources ranged between approximately 43% in ground beef and pork chops to around 83% in ground turkey in 2014 (Figure 19). There was a general decline since 2007 in *E. coli* prevalence among all meat sources except pork. The prevalence of *E. coli* cultured from animal cecal samples (Figure 20) ranges between 96% in turkey and 86% in beef cows.

![Figure 19. Prevalence of *E. coli* in Retail Meat Samples, 2002-2014](image)
Antimicrobial Resistance in Escherichia coli

In 2014, *E. coli* showing resistance to at least one antimicrobial were most common among turkey sources (83% from retail ground turkey meat and 90% from turkey ceca) and least frequent in isolates from cattle sources (23% from retail ground beef samples, 40% from beef cow ceca and 24% from dairy cow ceca).

Fluoroquinolone resistance
As with *Salmonella*, ciprofloxacin resistance in *E. coli* from retail meats, PR/HACCP chicken and cecal samples from chicken, turkeys, beef cows and dairy cows, market swine and sows was absent or low (0% to 0.7%).

Cephalosporin Resistance
Ceftriaxone resistance in *E. coli* isoates cultured from retail chicken meat decreased from a peak of 13% in 2011 to 6.6% in 2014. Retail turkey isolates showed a continued decline in ceftriaxone resistance during the same time period (from a peak of 10% in 2011 to 4.3% in 2013). As noted above, this coincided with declines in ceftriaxone resistance among nontyphoidal *Salmonella* from retail poultry meats.
Multidrug Resistance

In 2014, turkey sources had the highest prevalence of resistance to 3 or more drug classes (54% of retail ground turkey meat and; 66% of turkey cecal isolates were MDR), followed by isolates from retail chicken meat (36%), market swine (22%) and dairy cows (12%). The proportion of retail meat and cecal isolates exhibiting ACSSuT and ACSSuTAuCx resistance patterns was either absent or low.

Extremely Drug Resistant (XDR) E. coli

In 2014, there were no XDR resistant isolates from retail meat samples. From the cecal samples, there was one XDR isolate from a dairy cow that was resistant to all drug classes except β-lactam/β-lactamase inhibitor combinations.

Enterococcus

*Enterococcus* bacteria are naturally found in both animal and human intestinal microflora and are an indicator of fecal contamination in food. *Enterococcus* bacteria can be present in high numbers in food but they are not considered a major foodborne pathogen. Antimicrobial resistance in *Enterococcus* species is monitored to understand how resistance occurs in gram-positive bacteria.

As with *E. coli*, the NARMS Integrated Report includes data on *Enterococcus* bacteria in retail meat samples and food animal ceca but not from human sources. The two species most commonly recovered are *Enterococcus faecalis* and *Enterococcus faecium*. Results are reported by species because they differ in their ability to acquire and express resistance to various antimicrobials.

Prevalence of Enterococcus

*Enterococcus* is commonly recovered from retail meats, ranging in prevalence from 86% to 98% across commodities with no clear trends over time (Figure 21). The advent of cecal sampling in 2013 marked the first year that *Enterococcus* was recovered from all four food animal species at slaughter. Similar to 2013 findings, the majority (33%, 360/1089) of the 2014 *Enterococcus* isolates were *E. faecalis*, with the exception of beef cecal and dairy cecal samples where the majority of isolates were *E. hirae*. The prevalence of *Enterococcus* from the animal cecal samples is shown in Figure 22.
Figure 21. Prevalence of *Enterococcus* in Retail Meat Samples, 2002-2014

[Graph showing prevalence of Enterococcus in retail meat samples from 2002 to 2014 for chicken, ground turkey, ground beef, and pork chops.]

Figure 22. Prevalence of *Enterococcus* in Animal Cecal Samples, 2014

[Bar chart showing prevalence of Enterococcus in animal cecal samples for chickens, turkeys, cattle, dairy, market swine, and sows.]

Number of Enterococcus
Number samples tested

- Chickens: 100/112
- Turkeys: 60/63
- Cattle: 406/482
- Dairy: 227/261
- Market Swine: 166/193
- Sows: 130/147
**Antimicrobial Resistance in Enterococcus Isolates**

In samples collected in 2014, the majority (>79%) of *E. faecalis* and *E. faecium* isolates from all sources were resistant to at least one antimicrobial class. The retail poultry isolates were more likely to exhibit resistance to at least one antimicrobial class than were isolates from ground beef or pork chops.

The aminopenicillin, ampicillin, alone or in combination with an aminoglycoside, is a treatment of choice for susceptible *E. faecalis* infection. Other treatment options for enterococci infections include vancomycin, daptomycin, and linezolid. Resistance to these three antibiotics is generally very low (<2%) in *E. faecalis* isolates from all NARMS sources. In 2014, high-level gentamicin resistance (≥ 1000 mg/L) in *E. faecalis* isolated from retail chicken was 27%; a slight increase from 2002 (22%) when testing began. In ground turkey meat, 34% of isolates were gentamicin-resistant, which is 12 percent higher than 2002 levels (22%).

Vancomycin resistant *Enterococcus bacteria* has not been detected by NARMS in any retail food, PR/HACCP sample or animal cecal isolate. Linezolid resistance was detected in a single isolate of *E. faecalis* recovered from a beef cecal sample in 2014. Daptomycin is a lipopeptide antimicrobial used in the treatment of systemic and life-threatening infections caused by gram-positive organisms, including *Enterococcus faecalis*. In 2014, 4 *E. faecalis* isolates, 2 from beef cow ceca and 2 from swine (1 from a cecal sample, 1 from a retail pork chop samples) were resistant to daptomycin.

**Multidrug Resistance**

MDR has been consistently high among *E. faecium* and *E. faecalis* isolated from retail poultry. MDR in *E. faecium* from ground beef and pork chops has been less frequent (< 30% since 2005) with no significant trend. Cecal isolates of *E. faecium* and *E. faecalis* from swine tended to exhibit much higher levels of MDR, when compared to retail pork.

**References**


