Methods

The National Antimicrobial Resistance Monitoring System: Enteric Bacteria

Sampling

NARMS isolates originate from three distinct sources, which are listed below.

Surveillance of Human Clinical Isolates

The human component of NARMS was launched in 1996 within the framework of CDC's Emerging Infections Program and the Foodborne Diseases Active Surveillance Network (FoodNet). Initially, it included non-Typhi *Salmonella* and *Escherichia coli* O157 isolates from 14 state and local health departments. In 1999, *Salmonella* serotype Typhi and *Shigella* testing was added. By 2003, NARMS conducted nationwide surveillance of *Salmonella, Shigella*, and *E. coli* O157 from humans. Testing of *Campylobacter* from humans began in 5 FoodNet sites in 1997 and by 2003 expanded to all 10 FoodNet sites (California, Connecticut, Colorado, Georgia, Maryland, Minnesota, New Mexico, New York, Oregon, and Tennessee). In 2009, NARMS began testing *Vibrio* species other than *V. cholerae* from all 50 states. Sampling of human enteric pathogens is based on the occurrence of laboratory-confirmed cases of infection. Hospital based and private clinical laboratories isolate and identify enteric bacteria from human samples according to accredited methods.

Participating public health laboratories serotype the isolates before shipping to CDC for susceptibility testing. From 1996 through 2002, participating sites submitted every 10th non-Typhi *Salmonella* isolate they received to CDC for antimicrobial susceptibility testing[1]. From 2003 through 2007, sites submitted every 20th non-Typhi *Salmonella* isolate. Since 2008, they have submitted every 20th nontyphoidal *Salmonella* isolate.

From 1997 through 2004, CDC received the first *Campylobacter* isolated each week by each participating FoodNet laboratory. In 2005, a surveillance scheme for selecting a more representative sample of isolates was implemented. FoodNet sites changed to submitting every isolate (Connecticut, Georgia, Maryland, New Mexico, Oregon, and Tennessee), every other isolate (California, Colorado, and New York), or every fifth isolate (Minnesota) received. In 2010, the scheme for isolate submission was adjusted to every other isolate from Georgia and Maryland and every third from New Mexico.

[1] *Salmonella* serotype Paratyphi B is included in the nontyphoidal *Salmonella* sampling scheme because available laboratory methods do not always allow for distinction between serotype Paratyphi B (which typically causes typhoidal illness) and serotype Paratyphi B var. L(+) tartrate+ (which typically causes non-typhoidal illness). Only serotype Paratyphi B isolates that have been determined to be tartrate positive (Paratyphi B var. L(+) tartrate+) are included with non-typhoidal *Salmonella* for reporting purposes.

Antimicrobial susceptibility testing of NARMS human isolates is performed at CDC's laboratories in the National Center for Emerging and Zoonotic Infectious Diseases in Atlanta, Georgia.

Surveillance of Retail Meat Isolates

Retail meat surveillance is conducted by FDA through collaboration with 10 FoodNet sites, state departments of public health in Louisiana, Missouri, Pennsylvania, South Carolina, Washington, and universities in Iowa, Kansas, South Dakota, and Texas. When the retail program was launched in 2002, participating states included Connecticut, Georgia, Maryland, Minnesota, and Tennessee. Oregon joined the program in September of that year. Between 2003 and 2004, New York, California, Colorado, and New Mexico began conducting surveillance of retail meat isolates. Pennsylvania joined the program in 2008, and Missouri, Louisiana, and Washington joined in 2012. Most recently, Iowa, Kansas, South Dakota and Texas joined in 2016.

Sites select sampling zip codes that are at least within a 50 mile radius of their laboratories. NARMS identifies all grocery stores within these zip codes using the Chain Store Guide©. NARMS divides the states sampling areas into quadrants and uses a random number generator to randomly select the order that the quadrants are to be sampled. A randomized list of grocery stores is provided to sites each year. Stores are removed, updated as needed.Each month, participating laboratories purchase approximately 40 meat samples, including 10 samples each of retail chicken, ground turkey, ground beef, and pork chops. Prior to 2011, retail chicken was comprised of only chicken breast with bone and skin on, however in 2011 sites began sampling chicken wings, legs, and thighs when breast with bone in and skin on were unavailable.

Upon joining the program, all sites culture all meats for *Salmonella* and retail poultry for *Campylobacter*, however Pennsylvania did not begin testing for *Campylobacter* until one year after joining. Since 2008, sites have tested for *Campylobacter* in retail poultry only due to low isolation in previous years. Four sites (Georgia, Oregon, Maryland and Tennessee) culture all meat samples for *E. coli* and *Enterococcus*. Over the course of NARMS retail meat surveillance, some sites have declined testing for a period of a few months to a year due to competing public health priorities.

Sites isolate *Salmonella, Campylobacter, E. coli,* and *Enterococcus* from retail meats using methods described in the NARMS Retail Meat Isolation Protocol. Isolates are sent to the Food and Drug Administration (FDA) for species/serotype confirmation and antimicrobial susceptibility testing.

Surveillance of Food Animal Isolates

USDA's Agricultural Research Services (ARS) initiated the animal component of NARMS in 1997 by testing *Salmonella* isolates recovered by the USDA Food Safety Inspection Services (FSIS) Pathogen Reduction/Hazard Analysis and Critical Control Point (PR/HACCP) verification testing program. In the PR/HACCP program, *Salmonella* isolates were recovered from carcass rinsates (chicken), carcass swabs (turkey, cattle, and swine), and ground products (chicken, turkey, and beef) from federally inspected slaughter and processing plants throughout the United States. Of note, the USDA FSIS suspended cows/bulls sampling in 2011 and market hogs and steer/heifers in 2012 because of the low numbers of

positive samples. In 2014 *Salmonella* isolates were recovered from carcass rinsates (chicken), carcass swabs (turkey), ground/comminuted products (chicken and turkey), ground beef and beef trimmings.

Sampling methods used by FSIS for the PR/HACCP *Salmonella* verification testing program have changed since NARMS animal testing began. Before June 2006, there were two phases of the FSIS regulatory program for *Salmonella* in raw products: non-targeted and targeted testing. Non-targeted or "A" set samples were collected at establishments randomly selected from the population of eligible establishments, with a goal of scheduling every eligible establishment at least once a year. Other sample sets (e.g., "B", "C", and "D") were collected from establishments targeted for follow-up testing when HACCP performance standards were not met. *Salmonella* isolates from all the sets were included in NARMS testing, but most isolates were from “A” set samples. Beginning in June 2006, establishment testing was scheduled using risk-based criteria designed to focus FSIS resources on establishments with higher *Salmonella* positive results and also those with recovery of serotypes of public health importance. In 2013, continuous sampling of comminuted chicken and turkey was implemented with the purpose of developing a pathogen reduction performance standard for *Salmonella* in these products. Included in the NARMS Integrated Report are *Salmonella* isolates derived from this sampling in addition to those obtained from the risk-based sampling programs. Additional changes in sampling occurred in June of 2014 when FSIS began analyzing for *Salmonella* all raw beef samples collected for Shiga Toxin-Producing *Escherichia coli* (STEC) providing a non-risked based sample source. At the same time, *Salmonella* sampling set procedures in ground beef products was discontinued, except in establishments with results that exceeded the standard for *Salmonella* in their most recently completed set.

Since 1998, *Campylobacter* isolates recovered from chicken carcass rinsates were submitted to ARS from the Eastern laboratory for antimicrobial susceptibility testing. In 2011 all three FSIS laboratories began testing for and isolating *Campylobacter* from young chicken and young turkey carcasses through PR/HACCP verification sample sets at all eligible poultry slaughter establishments. In 2013, a new Cecal Sampling Program was launched by FSIS and FDA for food animal monitoring. Isolates of *Salmonella, Campylobacter, E. coli,* and *Enterococcus* described in the NARMS Integrated Report were recovered from the cecal contents of swine (market swine, sow), cattle (dairy cow, beef cow, steer, and heifer), young chicken, and young turkey in FSIS-regulated livestock and poultry slaughter establishments. Cecal sampling within slaughter establishments is scheduled at a frequency based on establishment size, animal classes slaughtered and annual slaughter volumes. Cecal samples from cattle and swine are form individual animals; chicken and turkey samples are composited from the ceca of five individual birds. Establishments are sampled in a tiered, randomized fashion based on slaughter volume, resulting in a scheme representative of overall national production.

Laboratory Methods

Once isolates are acquired from each surveillance component, they undergo further testing as described in the NARMS Interagency Laboratory Manual.

Data Analysis

Data Management

At CDC State partners enter isolate metadata, including bacterial identification data, electronically through the CDC NARMS Database web interface. CDC users uploaded antimicrobial susceptibility data and, when necessary, isolate species/serotype confirmation information for these isolates. All data were stored in an SQL Server database. Data were analyzed within the NARMS Database using SQL Server Reporting Services or exported to SAS for further analyses.

At FDA State metadata are recorded on log sheets submitted with the retail meat isolates. All data are stored in Microsoft Access and exported to SAS® and R® for analysis.

At USDA data were recorded in two locations. FSIS isolate information and metadata are electronically stored in the agency's Data Warehouse and LIMS. ARS laboratory data were stored in Microsoft Access and some records have been migrated into FSIS Data Warehouse and LIMS. For this report data were analyzed in (SAS® and WHONET 5.6).

Percent Resistant

The percentage of isolates resistant to any of the antimicrobials was defined as the number of isolates resistant to each antimicrobial divided by the total number of isolates tested for each antimicrobial. Antimicrobial resistance was defined according to the breakpoints listed with the NARMS Data Tables. All isolates with MICs that fall under the resistance breakpoint are considered to have no resistance detected. The total number of isolates with specific drug class resistance patterns (e.g. ≥ 3 antimicrobial classes) were calculated by summing the number of resistant isolates in each antimicrobial class. If an isolate was resistant to at least one drug in the class then it was resistant to the entire antimicrobial class. Starting in 2011, susceptibility testing included the macrolide azithromycin, resulting in nine antimicrobial classes for *Salmonella* and *E. coli*. Because resistance to azithromycin is <1%, antimicrobial class resistance, MDR data from 2011 and beyond are comparable to data from previous years. The total number of isolates drug-specific resistance profiles (e.g. 'At Least ACSSuT Resistant') were calculated by summing isolates that were resistant to all of the drugs in the profile. These isolates may have also had additional resistance to other antimicrobials (hence the term 'At Least').

Changes in Antimicrobial Resistance

Human Clinical Isolates

Using logistic regression, annual data from 2004–2014 was modelled to assess changes in the prevalence of antimicrobial resistance among *Salmonella* and *Campylobacter* isolates from humans. The prevalence of selected resistance patterns among isolates tested in 2014 was compared to the average prevalence from two reference periods, 2004–2008 and the previous five years, 2009–2013. The 2004–2008 reference period begins with the second year that all 50 states participated in *Salmonella* surveillance and all 10 FoodNet sites participated in NARMS *Campylobacter* surveillance. The additional 2009–2013 reference period allows for comparisons with more recent years.

In the logistic regression analysis for main effects, year was modelled as a 10-level categorical variable. To account for site-to-site variation in the prevalence of antimicrobial resistance, adjustments for site were included. The final regression models for *Salmonella* adjusted for the submitting site using the nine division categories described by the U.S. Census Bureau: East North Central, East South Central, Mid-Atlantic, Mountain, New England, Pacific, South Atlantic, West North Central, and West South Central. For *Campylobacter*, the final regression models adjusted for the submitting site using the 10 FoodNet states. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using unconditional maximum likelihood estimation. Ors were reported with 95% CIs (for 2014 compared with 2004–2008 and 2009–2013) that did not include 1.0 as statistically significant. More details on the methods and results from human surveillance are available in the CDC NARMS 2014 annual report.

Retail Meat Isolates

The Mann and Kendall methods were applied to NARMS retail meat isolates collected from 2002 through 2014. The analysis assesses the direction of the trend, the significance of a monotonic trend of microbial resistance over time, and rate of change in resistance. The results are comprised of the p-value (testing the significant of the trend), score (an indication of the direction of the trend), and rate (the magnitude of the change in resistance). The analysis was performed using R® version 3.3 and the results are covered in the NARMS Trend Analysis.

We applied the test considering the following antimicrobial/bacterium combinations:

- *Salmonella*: Ceftriaxone, Gentamicin, ACSSuT (ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline) **,** and the combination of at least three classes of antimicrobials
- *Campylobacter*: Erythromycin, Ciprofloxacin, and Gentamicin
- *E. coli*: Ceftriaxone, Ciprofloxacin, Azithromycin, Gentamicin, and the combination of at least three classes of antimicrobials
- E*nterococcus*: Linezolid, Daptomycin, Gentamicin, Vancomycin, and Penicillin

Direction of Trend

Assume $y_1, y_2, y_3, \ldots, y_n$ are proportions of antimicrobial resistance observed at time 1 to time n. The Mann and Kendall test statistic computes the difference between the later observed values and all earlier observed values, $y_j - y_i$, where y_j and y_i are measurements obtained at time j and i respectively where j > i. The difference is assigned to 1, 0, -1 for positive differences, no differences, and negative differences, respectively. A score S, indicating the direction of the trend (if any) is computed as:

$$S = \sum_{k-1}^{n-1} \sum_{j-k+1}^{n} (y_j - y_k)$$

A positive score indicates that observations obtained later in time tend to be larger than observations collected earlier whereas a negative score indicates that observations recorded later in time tend to be smaller than observations recorded earlier.

Significance of Trend

We tested the hypotheses: H_0 : No monotonic trend exists versus H_a : A monotonic trend exists

For $n \leq 10$, the probability table is used to look up S. If this probability is less than Alpha (α : Type one error probability) then the null hypothesis is rejected. Data shows evidences to support existence of the trend.

If $n > 10$

The test statistic, Z, is given by:

- If S is positive then $Z = \frac{S-1}{\sqrt{Var(S)}}$
- If S is negative then $Z = \frac{S+1}{\sqrt{Var(S)}}$
- If S=0 then $Z = 0$

$$VAR(S) = \frac{1}{18}\left[n(n-1)(2n+5) - \sum_{p-1}^{g} t_p(t_p-1)(2t_p+5) \right]$$

Where g the number of is tied groups and t_p is the number of observations in the p^{th} group.

The critical values are $Z_{1-\frac{\alpha}{2}}$. Reject the null H_0 if the test statistics is greater than the critical value.

Rate of Change

The rate of change in microbial resistance is calculated using the Sen slope estimator given by:

$\beta = median\left(\frac{y_j - y_i}{x_j - x_i}\right)$, for all i < j and i = 1, 2, …, n-1 and j = 2, 3,…, n

References:

1. Fleiss JL, Levin B, Paik MC. Statistical methods in for rates and proportions. In: Shewart WA, Wilks SS, eds. Wiley Series in Probability and Statistics. Published Online; 2004:284–308.

2. Kleinbaum DG, Kupper LL, Nizam A, Muller KE. Applied Regression Analysis and Other Multivariable Methods, 4th ed. Belmont. CA: Duxbury; 2008.