Methods

The National Antimicrobial Resistance Monitoring System: Enteric Bacteria

Sampling

NARMS isolates originate from three distinct sources, which are described 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 (CA), Connecticut (CT), Colorado (CO), Georgia GA), Maryland (MD), Minnesota (MN), New Mexico (NM), New York (NY), Oregon (OR), and Tennessee (TN)). 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 perform whole genome sequencing (WGS) and serotype the isolates before shipping to CDC for susceptibility testing. As with the other NARMS components (see below), partner labs submit the WGS data to NCBI for public access. 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. CDC matches the WGS information submitted by the public health laboratories with susceptibility results and use these data for NARMS reporting.

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 (CT, GA, MD, NM, OR, and TN), every other isolate (CA, CO, and NY), or every fifth isolate (MN) received. In 2010, the scheme for isolate submission was adjusted to every other isolate from GA and MD and every third from NM. 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 15 state public health

[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.

laboratories (CA, CO, CT, GA, LA, MD, MN, MO, NM, NY, OR, PA, SC, TN, WA) and 7 universities (University of California at Davis (UCD), Iowa State University (IA), Kansas State University (KS), North Carolina State University Raleigh (NC) , Ohio State University (OH), South Dakota State University (SD) and Texas Tech University (TX)) . 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. IA, KS, SD and TX joined in 2016. UCD and NC joined in 2017 and Ohio in 2019.

In 2021, FDA shifted to from convenience sampling to a population based stratified double clustering design. According to the US Census Bureau, the US is divided into 4 major regions: West, Midwest, Northeast, South. Within each region, the population is grouped into LARGE (>10,000,000), MEDIUM (10,000,000 >= population> 5,000,000), and SMALL (<=5,000,000) clusters. Within each cluster, the population is furthermore divided into urban or rural areas, then into zip codes. Based on population density, for a LARGE cluster, at least 3 cities and at least 2 rural areas are selected; for a MEDIUM cluster, at least 2 cities and at least 1 rural area are selected; and for a SMALL cluster, at least 1 city cluster and at least 1 rural cluster are selected. The list of all valid zip codes from selected cities/rural areas is used to randomly assign zip codes from which retail meats are weekly/ quarterly purchased for cities or rural areas, respectively.

Each month, participating laboratories purchase approximately 30 meat samples, including 8 samples each of retail chicken, ground turkey, ground beef/pork chops (sampling years alternate), 2 samples each of salmon, tilapia and shrimp. For retail chicken, sites have the option to purchase chicken wings, legs, thighs, whole chicken or mixed parts when breast with bone in and skin on were unavailable. When ground turkey is not available, sites may purchase ground turkey breast or turkey patties. Starting in 2019, sites began sampling ground pork and only purchase pork chops when ground pork is 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, only retail poultry has been tested for *Campylobacter* in retail poultry only due to low isolation in retail ground beef and pork in previous years. For all retail meats, 16 sites (UCD[2],CO,CT,GA, KS, MD,NC, OH,OR,PA,SC,SD[3],TN, TX[4]) culture for *E. coli* and 14 sites (UCD,CT,GA,KS,MD,NC,OH,OR,SC,SD,TN,TX) culture for *Enterococcus*. Beginning in 2020, NARMS retail meat sites began collecting seafood (salmon, tilapia, and shrimp) and culturing them for *Enterococcus*, *Aeromonas*, and *Vibrio*. 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 and Seafood Isolation Protocols. *Salmonella*, *Campylobacter*, and *E. coli* isolates are subjected to WGS and the data are sent to the NCBI. Isolates are then sent to the Food and Drug Administration (FDA) for species/serotype confirmation and antimicrobial susceptibility testing.

[2] UCD collects retail samples in Los Angeles County and Honolulu
[3] SD also collects retail samples in ND
[4] TX also collects retail samples in Oklahoma City

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 as well as 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.

Animal isolates are tested for antimicrobial susceptibility and subjected to WGS at the FSIS laboratory. Additional information on FSIS testing can be found here.

Laboratory Methods

Details on the laboratory processes and tests performed in NARMS is 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®,R®, and Tableau® 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®,WHONET, and Tableau ®).

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. Beginning in 2021, all drugs tested (both historical and current) are considered when determining resistance to an antimicrobial class. This ensures that historical isolates that were reported as resistant to a class of antimicrobial continue to be reported as resistant to that class even if a drug is removed from the testing panel.

Whole Genome Sequencing

The identification of known antimicrobial resistance genes from whole genome sequencing data correlates very highly (>95% for most drugs) with isolates exhibiting MICs above the clinical breakpoint. Therefore, WGS can be used to accurately predict resistance from genotype alone. Since WGS can be performed quickly, predicted resistance based on genomic sequences is the primary data set in NARMS for real time reporting. At FDA, state partners sequence isolates from retail meat sources using either the CDC PulseNet or FDA Genometrakr sequencing method. The sequences generated are analyzed to meet program quality standards and are submitted to the National Center for Biotechnology Information (NCBI) along with relevant metadata. At NCBI, algorithms identify resistance genes which are then compared to antimicrobial susceptibility testing (AST) results to confirm results.