

National Antimicrobial Resistance Monitoring System Retail Meat Surveillance Laboratory Protocol

Objective:

The goal of this study is to determine the prevalence of antimicrobial resistance among *Salmonella*, *Campylobacter*, *E. coli* and Enterococci isolated from retail samples of retail chicken, ground turkey, ground beef and pork chops purchased from grocery stores in the United States.

Methods

Sample collection:

Purchase a total of 80 food samples per month including 40 samples of retail chicken¹ (skin-on/bone-in), 20 ground turkey, 10 ground beef (~80% lean) and 10 pork chops. For retail chickens, 2 to 4 of the samples collected must be organic. Record the demographic information for each sample including store name and location, brand name, sell-by date, purchase date and lab processing date for each purchased meat and poultry sample on the monthly log sheets. Record whether the meat or poultry sample packaged in the store and the packaging type². If the information is provided on the meat package, record the country(ies) of origin. Keep samples on ice during transport from the grocery stores to the laboratory. Refrigerate samples at 4°C and begin laboratory processing within 96 hours after purchase.

Processing Day 1:

Do not open packages until ready to begin processing. Place intact packages of meat and poultry samples on a clean surface and aseptically open. Ensure external surface and edges of wrappings do not touch meat and poultry samples. Remove meat or poultry sample with sterile instruments (e.g., tongs, gloves, or spoons). Each site should photocopy all meat and poultry package labeling and file copies for future reference.

Aseptically place one retail chicken breast with bone-in/skin-on from each package of chicken into a sterile plastic bag (e.g., stomacher bag). More than one piece may be used to achieve a 25 g sample when substituting chicken breast with another bone-in/skin-on chicken part. Aseptically place one pork chop from each package of pork into a sterile plastic bag. Aseptically place 25 g ground turkey from each package of ground turkey into a sterile plastic bag. Aseptically place 25 g ground beef from each package of ground beef into a sterile plastic bag. By repeating this process for each package of meat and poultry, there should be one bag for each meat and poultry sample. Add 225 ml buffered peptone water to each bag. Shake bags manually for 3 minutes or mix on a mechanical shaker at 200 rpm for 15 minutes.

¹ When bone-in/skin-on chicken breasts are not available for sampling, chicken wings are the preferred substitution followed by drumsticks and chicken thighs.

² Examples of the packaging types located in Appendix A (p.7).

Aseptically transfer 50 ml rinse from each bag into a flask (or other suitable sterile container). This flask will be used for *Salmonella* isolation. Aseptically transfer another 50 ml rinse from each bag into a second sterile container. This container will be used for *Campylobacter* isolation. For sites doing *E. coli* and Enterococcus, an additional 50 ml rinse from each bag will be transferred into each of two additional flasks, one for *E. coli* isolation and one for enterococci isolation.

Note: For *Campylobacter*, only set up retail chicken and ground turkey samples. Ground beef and pork chops should NOT be set up due to their low prevalence of *Campylobacter*.

Salmonella

Add 50 ml double strength (2X) lactose broth to each flask of 50 ml rinse used for *Salmonella* isolation. Mix thoroughly and incubate at 35°C for 24 hours.

Campylobacter

Add 50 ml double strength (2X) Bolton broth to each container of 50 ml rinse used for *Campylobacter* isolation. Mix thoroughly, but gently to avoid aeration, and incubate containers (with cap loose) in a microaerophilic atmosphere (85% N₂, 10% CO₂, 5% O₂) or in a jar with a CampyPak for 24 hours at 42°C.

E. coli

Add 50 ml double strength (2X) MacConkey broth to each flask of 50 ml rinse used for *E. coli* isolation. Mix thoroughly and incubate at 35°C for 24 hours.

Enterococci

Add 50 ml double strength (2X) enterococcosel broth to each flask of 50 ml rinse used for enterococci isolation. Mix thoroughly and incubate at 45°C for 24 hours.

Processing Day 2:

Salmonella

Transfer 0.1 ml from one flask to a test tube containing 9.9 ml RVR10 (Rappaport-Vassiliadis) medium; repeat for each flask. Incubate test tubes of RVR10 medium in water bath at 42°C for 16-20 hours.

Campylobacter

Dip a swab into one container and swab the first quadrant of a Campy Cefex Agar (CCA) plate. Using a loop, streak the remainder of the plate to obtain isolated colonies. Repeat procedure for each container. Incubate the plates in a microaerophilic atmosphere (85% N₂, 10% CO₂, 5% O₂) or in a jar with a CampyPak at 42°C for 24 hr.

E. coli

Streak one loopful from one flask onto the first quadrant of a Maconkey (MAC) agar plate. Then, streak the remainder of the plate to obtain isolated colonies. Repeat procedure for each flask. Incubate plates at 35°C overnight.

Enterococci

If no growth or blackening is observed in a flask, sample is negative and can be discarded; complete

the log sheet and place a check mark under the no growth column. If growth and blackening is observed in the flask, streak one loopful onto the first quadrant of an Enterococcosel agar plate. Then, streak the remainder of the plate to obtain isolated colonies. Repeat procedure for each flask. Incubate plates at 35°C for 24 hours.

Processing Day 3:

Salmonella

For each RVR10 culture, pre-warm to 35-37°C a 10 ml tube of M Broth. Transfer 1 ml from the incubated RVR10 cultures to the pre-warmed tubes of M Broth. Refrigerate the remaining portion of each RVR10 culture at 4-8°C. Incubate the inoculated M Broth tubes in a water bath at 35-37°C for 6-8 hours. Heat 1 ml of each incubated M Broth culture at 100°C for 15 minutes and refrigerate the remaining portion of each M Broth tube at 4-8°C*. Cool the heated 1 ml portion from each M Broth culture to room temperature and test immediately* using the TECRA VIA *Salmonella* kit (or the VIDAS kit used according to instructions). If the TECRA VIA is negative, discard the M Broth and RVR10 cultures; complete the log sheet and place a check mark under the no growth column. If the TECRA VIA is positive, streak one loopful of the unheated M Broth culture onto a xylose lysine desoxycholate (XLD) agar plate and streak one loopful of the RVR10 culture onto a second XLD agar plate, streaking to obtain isolated colonies. Repeat for each positive culture. Incubate the plates at 35°C overnight.

**Optional:* After heating the 1 ml portion of M broth at 100°C for 15 minutes, refrigerate it overnight at 4-8°C and then proceed as described above, beginning with “test immediately using the TECRA VIA *Salmonella* kit...”, etc.

Campylobacter

Examine each CCA plate for typical *Campylobacter* colonies (round to irregular with smooth edges; thick translucent white growth to spreading, film-like transparent growth). If typical growth is present, select one typical, well-isolated colony and streak for isolation onto a blood agar plate (BAP). Incubate the BAP in a microaerophilic atmosphere (85% N₂, 10% CO₂, 5% O₂) or in a jar with a CampyPak at 42°C for 24 hours. Repeat procedure for each CCA plate. If a CCA plate does not have any typical colonies, re-incubate the plate as previously described.

E. coli

Examine each MAC plate for typical *E. coli* colonies (pink colonies). If no typical growth is observed on MAC agar plate, sample is negative and can be discarded; complete the log sheet and place a check mark under the no growth column. If typical growth is present, select one typical, well-isolated colony and streak for isolation onto a BAP. Repeat procedure for each MAC plate. Incubate BAP(s) at 35°C overnight.

Enterococci

Examine each enterococcosel agar plate for typical enterococcal colonies (surrounding medium black). If no typical growth is observed on the enterococcosel agar plate, sample is negative and can be discarded; complete the log sheet and place a check mark under the no growth column. If typical growth is present, select one typical, well-isolated colony and streak for isolation onto a BHI (or other non-blood containing) agar plate. Repeat procedure for each enterococcosel agar plate. Incubate BHI plate(s) at 35°C for 24 hours.

Processing Day 4:

Salmonella

Examine each XLD agar plate for typical *Salmonella* colonies (pink colonies with or without black centers; colonies may have large, glossy black centers or may appear as almost completely black colonies). If no typical growth is observed on XLD agar plate, sample is negative and can be discarded; complete the log sheet and place a check mark under the no growth column. If typical growth is present, select one typical, well-isolated colony and streak for isolation onto a BAP. Repeat procedure for each XLD agar plate. Incubate BAP(s) at 35°C overnight.

Campylobacter

For blood agar plates: Examine each blood agar plate for purity and typical *Campylobacter* colonies (round to irregular with smooth edges; thick translucent white growth to spreading, film-like transparent growth). If growth is pure and colonies are typical, perform a Gram stain and an oxidase, catalase and motility test (hippurate optional) to confirm growth as *Campylobacter*. If there is no typical growth on blood agar plate or no colonies are positive for *Campylobacter* (Gram-negative rods with corkscrew motility, catalase positive, oxidase positive), sample is negative and can be discarded; complete the log sheet and place a check mark under the no growth column. If positive for *Campylobacter* and hippurate positive, record on log sheet as *C. jejuni*. If positive for *Campylobacter* and hippurate negative, use PCR testing to confirm as *C. coli*. (Note: PCR is optional and may be used in addition to or in place of a hippurate test to identify an isolate as *C. jejuni* or *C. coli*.) Repeat procedure for each blood agar plate. If an isolate is positive for *Campylobacter* but cannot be confirmed as *C. jejuni* and *C. coli*, freeze at -60 to -80°C in Brucella broth with 15% glycerol mixture. Ship all isolates in a cryovial on dry ice to FDA-CVM. Laboratories should keep duplicates of strains within their culture collections.

For CCA plates: Examine each re-incubated CCA plate for typical colonies (round to irregular with smooth edges; thick translucent white growth to spreading, film-like transparent growth). If no typical growth is observed, sample is negative and can be discarded; complete the log sheet and place a check mark under the no growth column. If typical growth is present on CCA plate, select one typical, well-isolated colony and streak for isolation onto a BAP. Repeat procedure for each CCA plate. Incubate BAP(s) in a microaerophilic atmosphere (85% N₂, 10% CO₂, 5% O₂) or in a jar with a CampyPak at 42°C for 24 hours.

E. coli

Examine each blood agar plate for purity and typical *E. coli* colonies. If no typical growth is observed, sample is negative and can be discarded; complete the log sheet and place a check mark under the no growth column. If typical growth is observed, perform an indole test (oxidase optional) on each BAP. If the growth is pure, indole positive (oxidase negative), swab the growth into Brucella broth with 15% glycerol mixture and freeze at -60 to -80°C. Repeat procedure for each blood agar plate. Ship all isolates in a cryovial on dry ice to FDA-CVM. Laboratories should keep duplicates of strains within their culture collections.

Enterococci

Examine each BHI agar plate for purity and typical enterococcal colonies. If no typical growth is

observed, sample is negative and can be discarded; complete the log sheet and place a check mark under the no growth column. If typical growth is observed, Gram stain the suspected colonies. If the Gram stain is atypical, sample is negative for enterococci and can be discarded; complete the log sheet and place a check mark under the no growth column. If Gram-positive cocci are observed, perform a catalase test. If catalase negative, confirm further with a PYR test. If catalase positive or PYR negative, plates may be discarded; complete the log sheet and place a check mark under the no growth column. If results produce catalase negative and PYR positive, record the isolate as *enterococcus*. Repeat procedure for each BHI agar plate. Sites should freeze each *enterococcus* isolate at -60 to -80°C in Brucella broth with 15% glycerol mixture and ship all isolates in a cryovial on dry ice to FDA-CVM. Laboratories should keep duplicates of strains within their culture collections.

Processing Day 5:

Salmonella

Examine each BAP for purity. If pure, confirm as *Salmonella* by standard biochemical methods, API or VITEK, and serotype (optional). Repeat for each blood agar plate. Sites should freeze each *Salmonella* isolate at -60 to -80°C in Brucella broth with 15% glycerol mixture and ship all isolates in a cryovial on dry ice to FDA-CVM. Laboratories should keep duplicates of strains within their culture collections.

Campylobacter

Examine each blood agar plate for purity and typical *Campylobacter* colonies (round to irregular with smooth edges; thick translucent white growth to spreading, film-like transparent growth). If growth is pure and colonies are typical, perform a Gram stain and an oxidase, catalase and motility test (hippurate optional) to confirm growth as *Campylobacter*. If there is no typical growth on blood agar plate or no colonies are positive for *Campylobacter* (Gram-negative rods with corkscrew motility, catalase positive, oxidase positive), sample is negative and can be discarded; complete the log sheet and place a check mark under the no growth column. If positive for *Campylobacter* and hippurate positive, record on log sheet as *C. jejuni*. If positive for *Campylobacter* and hippurate negative, use PCR testing to confirm as *C. coli*. (Note: PCR is optional and may be used in addition to or in place of a hippurate test to identify an isolate as *C. jejuni* or *C. coli*.) Repeat procedure for each blood agar plate. If an isolate is positive for *Campylobacter* but cannot be confirmed as *C. jejuni* and *C. coli*, freeze at -60 to -80°C in Brucella broth with 15% glycerol mixture and ship all isolates in a cryovial on dry ice to FDA-CVM for speciation and antimicrobial susceptibility. Laboratories should keep duplicates of strains within their culture collections.

Preparing the Isolates:

Collect each isolate from the plate. Each culture plate should yield only one isolate. Sites should freeze each *Salmonella*, *Campylobacter*, *E. coli* and enterococci isolate at -60 to 80°C in Brucella broth with 15% glycerol mixture. Record whether or not there was bacterial growth for each meat and poultry sample. Please label each tube with isolate ID# and organism. Isolate ID # should be the same as the isolate ID# on log sheet. Laboratories should keep duplicates of strains within their culture collections until notified by FDA-CVM that the cultures were pure and viable after shipment and the duplicates may be discarded.

Packaging the Isolates:

Ship all isolates in cryogenic vials wrapped with parafilm to keep tops from coming unscrewed. Cryogenic vials should be wrapped using bubble wrap, cotton, paper towels, etc. to ensure they do not break during shipping. Place cryogenic vials in a shipping container with plenty of dry ice placed in a box for shipping. Cryogenic vials should be shipped to FDA-CVM in accordance with current shipping of hazardous material guidelines. Prior to shipment of isolates to FDA-CVM, sites should e-mail a copy of the completed log sheets to NARMS retail study liaisons at both FDA and CDC. The original log sheets or hard copies of electronic log sheets should be included with each isolate shipment to FDA-CVM. Each site should retain copies of log sheets for their records.

Shipping the Isolates:

Packages should be sent **overnight** or priority mail with 1-2 day delivery. Please ship isolates so they will arrive at FDA-CVM by Thursday. If the isolates will not arrive by Thursday, please store them in your freezer and ship the following Monday morning. Shipments should occur once per month.