Changes to the retail meat protocol

2017

- Tecra Kit no longer used
- Started 4 colony picks for Salmonella- XLT4 and HE
- Sampling scheme for Enterococcus and E coli changed to
  - Every 8th retail chicken sample- 5 samples from each site
  - Every 5th ground turkey sample- 4 sample from each site
  - Every other ground beef and pork chop sample- 5 samples

2018

- Allowed ground turkey patty to be sampled if ground turkey unavailable

2019

- Salmonella isolation changed from 4 picks from two agars to two picks from XLT4 with sites only sending one isolate
- Increased ground meats from 25g to 50 g
- Overnight enrichment of Salmonella added to protocol
- For retail pork, required that sites collect ground pork samples primarily and pork chop when ground pork not available
Objective:

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

Sample collection:

Purchase a total of 80 food samples per month including 40 samples of retail chicken (bone-in/skin-on), 20 ground turkey, 10 ground beef \(^1\) (~80% or 85% lean) and 10 samples of retail pork (ground or chops). For retail chickens, please look for breasts. If you cannot find breasts, you may purchase thighs or wings. If you purchase whole chicken or mixed parts, please test the chicken breasts. If the above packages cannot be found, then you can sample legs. Two to four of the retail chicken samples must be organic. For retail pork, please sample ground pork. If you cannot find ground pork, then sample pork chops.

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 was packaged in the store and the packaging type \(^2\). 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. For ground beef, do not select meat that is higher than 85% fat. For ground turkey, any fat percentage is acceptable. If ground turkey is not available, select ground turkey breast and ground turkey patties which have not been previously frozen.

\(^1\) For ground beef, do not select meat that is lower than 75% or higher than 85% lean
\(^1\) Examples of the packaging types located in Appendix A
\(^1\) If ground turkey is not available, you may collect ground turkey breast or turkey patties not previously frozen.
\(^2\) Examples of the packaging types located in Appendix A (p.8).
Processing Day 1:

* Please note: Media should be brought to room temperature prior to inoculation for use on each day as needed below.

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

Set Up Process:

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 50 g sample when substituting chicken breast with another bone-in/skin-on chicken part. Aseptically place 50 g of ground pork into a sterile plastic bag or aseptically place one pork chop from each package of pork into a sterile plastic bag. Aseptically place 50 g ground turkey from each package of ground turkey into a sterile plastic bag. Aseptically place 50 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 250 ml buffered peptone water to each bag. For bone in samples, hand massage samples for 3 minutes or use a mechanical shaker at 200 rpm for 15 minutes. For ground meat products, stomach at low speed (230 rpm) for 90 seconds, hand massage for 3 minutes, or use a mechanical shaker at 200 rpm for 15 minutes until clumps are dispersed.

Enrichment Preparation:

Aseptically transfer 50 ml rinse from each bag into a separate sterile flask (or other suitable sterile container) for *Campylobacter*. For sites doing *E. coli* and/or *Enterococcus*, an additional 50 ml rinse from each bag will be transferred into separate sterile flasks.

*Salmonella*

Leave chicken parts and ground meats in the remaining Buffered Peptone Water (BPW) and incubate overnight at 35°C for 24 hours.

Notes:

If you are using the BAX or VIDAS system, please use the manufactures instructions to screen samples for Salmonella.

If you are using an in-house PCR method, it must be validated, and the method sent to FDA/CVM for approval prior to using the method for this surveillance study.
**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 or bags (with loosened caps or closed loosely) in a microaerophilic atmosphere (85% N2, 10% CO2, 5% O2) or in a jar with a CampyPak for 24 hours at 42°C.

**Notes:**
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_.

Do not stack containers. This will prevent some of the samples from achieving the appropriate aerophilic atmosphere.

**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.

**Enterococcus**
Add 50 ml double strength (2X) Enterococcusel broth to each flask of 50 ml rinse used for _Enterococcus_ 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 10 ml RVR10 (Rappaport-Vassiliadis) medium; repeat for each flask. Incubate test tubes of RVR10 medium in water bath at 42°C for 20-24 hours.

**Campylobacter**
Carefully mix 2X Bolton broth avoiding aeration, dip a saturated cotton swab into one container and swab the first quadrant of a Campy Cefex Agar (CCA) plate and discard. 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 hours.

**E. coli**
Streak one loopful from one flask onto the first quadrant of a MacConkey (MAC) 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.

**Enterococcus**
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, vortex or mix the enrichment before you streak to one XLT-4 plate, incubate at 35°C for 18-24 hours.

**Notes:**

If you have a positive from the BAX or VIDAS systems, streak the positive samples to XLT-4 agar and incubate at 35°C for 18-24 hours.

Optional Selective Agars: Chromogenic agar, HE agar, or a selective non-H2S producing agar for *Salmonella* may be used in conjunction with XLT4 to obtain a positive isolate. These suggested agars are not to be used to replace XLT-4 agar.

*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. Chromogenic agar may be used in conjunction with CCA in identifying *Campylobacter*. Indicate on the log sheet when you are not sending an isolate from the CCA plate.

*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, indicate results on log sheet for E. coli. If typical growth is present, select one typical, well-isolated colony and streak for isolation onto a blood agar plate. Repeat procedure for each MAC plate. Incubate blood agar plate at 35°C for 24 hours.

*Enterococcus*

Examine each Enterococcosel agar plate for typical *Enterococcus* colonies (surrounding medium black). If no typical growth is observed on the Enterococcosel agar plate, sample is negative and can be discarded; indicate results on log sheet for *Enterococcus*. 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 XLT-4 plate for typical *Salmonella* colonies. For XLT-4, look for pink colonies with or without black centers; colonies may have large, glossy black centers or may appear as almost
completely black colonies. If you have typical or atypical growth, pick two colonies from XLT-4. If typical growth is present, confirm that the two colonies are *Salmonella*. Once confirmed, please pick one colony for isolation onto a blood agar plate. Incubate blood agar plate at 35°C for 24 hours. Repeat for each sample. If no typical growth is observed on XLT-4, sample is negative and can be discarded; indicate results on log sheet for *Salmonella*.

**Campylobacter**

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; indicate results on log sheet for *Campylobacter*. If typical growth is present on CCA plate, select one typical, well-isolated colony and streak for isolation onto a blood agar plate. Repeat procedure for each CCA plate. Incubate blood agar plate in a microaerophilic atmosphere (85% N2, 10% CO2, 5% O2) or in a jar with a CampyPak at 42°C for 24 hours.

**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, oxidase and catalase (motility and hippurate tests are optional) tests 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 select no for *Campylobacter*. If hippurate test is performed and is positive, record on log sheet as *C. jejuni*. If positive for *Campylobacter* and hippurate negative, use PCR testing to confirm as *C. jejuni*. (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*). If *Campylobacter* positive and no speciation is done, record on log sheet as *Campylobacter* species. Repeat procedure for each sample blood agar plate. Freeze all *Campylobacter* positive isolates 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.

**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 select no for *E. coli*. If typical growth is observed, perform an indole test (oxidase optional) on each blood agar plate. If the growth is pure, and 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 on dry ice to FDA-CVM. Laboratories should keep duplicates of strains within their culture collections.

**Enterococcus**

Examine each BHI agar plate for purity and typical enterococci colonies. If no typical growth is observed, sample is negative and can be discarded; complete the log sheet and select no for *Enterococcus*. 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 select no for *Enterococcus*. 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 select no for Enterococcus. If results produce catalase negative and PYR positive, record the isolate as Enterococcus. Repeat procedure for each BHI agar plate. Subculture one well isolated colony from BHI to blood agar plate. Incubate at 35°C for 24 hours.

Processing Day 5:

Salmonella
Examine each BAP for purity. If pure, confirm as Salmonella by standard biochemical methods, API or VITEK, MALDI-TOF and serotype (optional). For sites that confirm and serotype two isolates and get 2 different serotypes from one meat sample, sequence both isolates and forward both isolates to CVM for further testing. 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 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). For those plates that were re-incubated from Day 3 that had low growth, recheck for positive growth and continue processing. 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 select no for Campylobacter. 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 on dry ice to FDA-CVM for speciation and antimicrobial susceptibility. Laboratories should keep duplicates of strains within their culture collections.

Enterococcus
Examine each blood agar plate for purity and typical Enterococcus growth. Sites should freeze each Enterococcus isolate at -60 to -80°C in Brucella broth with 15% glycerol mixture and ship all isolates on dry ice to FDA-CVM. Laboratories should keep duplicates of strains within their culture collections.

Whole Genome Sequencing
Please refer to PulseNet and GenomeTrakr sequencing protocols. Also, refer to NARM’s Sequencing Guidelines for additional instructions.

Preparing Isolates for Shipment:
Please label each vial with NARMS isolate ID. The NARMS isolate ID on the vial should match the
NARMS isolate ID on the log sheet. Laboratories should keep duplicates of strains within their culture collections until notified by FDA-CVM that the duplicates may be discarded (isolates can be discarded once the NARMS report for the testing year has been published).

Packaging the Isolates:
Ship all isolates in cryogenic vials with parafilm wrapped tops to keep tops from coming unscrewed. Cryogenic vials should be properly wrapped with absorbent material to prevent leakage during shipment. 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 FDA. 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. 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. Shipments must occur on a monthly basis. Send all shipments to Shawn McDermott at the following address:

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