National Antimicrobial Resistance Monitoring System
(NARMS)
Retail Meat Surveillance Laboratory Protocol

Objective:

Determine the prevalence of antimicrobial resistance of *Salmonella*, *Campylobacter*, *Escherichia coli* and *Enterococcus* isolates in retail meat and poultry samples. Samples of chicken, ground turkey, and ground beef[1] are purchased from grocery stores in the United States.

2021 Sample collection:

PRODUCT	NUMBER OF SAMPLES PER MONTH	SAL	CAMPY	E COLI	ENT	VIBRIO	AERO	LACTOSE FERMENTERS
Chicken Breast	8	8	4	8	8			
Ground Turkey	8	8	**DNT[2]**	8	8			
Ground Beef	**8**	**8**	**DNT**	**8**	**8**			
Shrimp	2				2	2	2	2
Tilapia	2				2	2	2	2
Salmon	2				2	2	2	2
Chicken Liver	1	1	1	1	1			
Chicken Gizzard	1	1	1	1	1			
Chicken Heart	1	1	1	1	1			
Total	**33**	**27**	**7**	**27**	**33**	**6**	**6**	**6**

Purchase a total of 33 food samples per month:

- 8 samples of retail chicken (bone-in/skin-on)
- 8 ground turkey (any fat percentage is acceptable)
- 8 ground beef [1] (~80% or 85% lean)
- 1 each of chicken liver
- 1 Chicken gizzard
- 1 Chicken heart
- 2 Shrimp
- 2 Tilapia

[1] For ground beef, do not select meat that is lower than 75% or higher than 85% lean
[2] DNT= DO NO TEST

- 2 Salmon

For seafood isolates, follow the seafood protocol.

Note: Pork chops or ground pork will not be collected for 2021 sampling.

For retail chicken samples, purchase breasts. If you cannot purchase breasts, purchase thighs or wings. If you purchase whole chicken or mixed parts, test the chicken breasts. If chicken breast, thighs, or wings cannot be found, purchase legs. One or two of the retail chicken samples must be organic or raised without antibiotics. **Purchase chicken giblets separated by type: liver, heart, and gizzard. If you cannot find chicken giblets separated by type, purchase package of mixed giblets. Do not test more than one type of giblets from the mixed package. If a whole chicken is purchased, with giblets stuffed inside, these giblets are considered as one chicken giblets package.**

Record demographic information for each purchased meat sample on the monthly log sheets. Demographic information must include: store name and location, brand name, sell-by date, purchase date, and lab processing date. Record whether the meat or poultry sample was packaged in the store and the packaging type. 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. If ground turkey is not available, select ground turkey breast and ground turkey patties which have not been previously frozen[3].

[3] If ground turkey is not available, you may collect ground turkey breast or turkey patties not previously frozen

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 test two *different types* of chicken giblets from the same package.

Do not store the retail meat samples on ice. 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. 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 (example: stomacher bag). More than one piece of chicken may be used to achieve a 50 g sample when substituting chicken breast with another bone-in/skin-on chicken part. **More than one piece of the chicken giblet may be needed to achieve a 50 g sample. If two pieces are needed, it must be two hearts, two livers or two gizzards. Aseptically** place 50 g of ground turkey from each package into a sterile plastic bag. Aseptically place 50 g of ground beef into a sterile plastic bag. Repeat 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, carefully hand massage samples for 3 minutes (being careful not to puncture the bag), 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 containers.

Salmonella

Leave chicken parts, chicken giblets 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, incubate containers or bags (caps loosened 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 samples. Ground beef should NOT be set up, due to their low prevalence of *Campylobacter*.

Ground turkey will not be tested for Campylobacter in 2021 sampling.

Do not stack containers. This will prevent samples from achieving the appropriate microaerophilic atmosphere.

E. coli

Add 50 ml double strength (2X) MacConkey broth to each container 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) Enterococcosel broth to each container 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 container of overnight BPW to a test tube containing 10 ml RVR10 (Rappaport-Vassiliadis) medium; repeat for each container. 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 cotton tip swab into one container until saturated and swab the first quadrant of a Campy Cefex Agar (CCA) plate. Discard swab. 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 container of 2X MacConkey broth onto the first quadrant of a MacConkey (MAC) agar plate. Streak the remainder of the plate to obtain isolated colonies. Repeat procedure for each container. Incubate plates at 35°C for 24 hours.

Enterococcus

If no growth or blackening is observed in a container, 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 container, streak one loopful onto the first quadrant of an Enterococcosel agar plate. Streak the remainder of the plate to obtain isolated colonies. Repeat procedure for each container that has growth *and* blackening. 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). **If the CCA plate does not have isolated colonies, streak again to another CCA before subculturing to 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 at 42°C for 24 hours. **Chromogenic agar may be used in conjunction with CCA in identifying *Campylobacter*. Indicate on the log sheet when you are using Chromogenic agar and sending an isolate, instead of 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, record 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. **If the MAC plate does not have isolated colonies, streak again to another MAC before subculturing to BAP.** Incubate blood agar plates at 35°C for 18-24 hours.

Enterococcus

Examine each Enterococcosel agar plate for typical *Enterococcus* colonies (brownish-black to black zones around colonies). If no typical growth is observed on the Enterococcosel agar plate, sample is negative and can be discarded; record 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**. If the Enterococcosel plate does not have isolated colonies, streak again to another Enterococcosel plate before subculturing to BHI.** 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 the XLT-4 plate does not have isolated colonies, streak again to another XLT-4 before subculturing to BAP.** 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 18-24 hours. Repeat for each sample. If no typical growth is observed on XLT-4, sample is negative and can be discarded; record results on log sheet for *Salmonella*.

Campylobacter

For re-incubated 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; record 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. **If the CCA plate does not have isolated colonies, streak again to another CCA before subculturing to BAP.** Repeat procedure for each CCA plate. Incubate blood agar plate(s) 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 tests to confirm growth as *Campylobacter* (motility and hippurate tests are optional). 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; recorded results on log sheet, 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. 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)*.** If *Campylobacter* positive and no speciation is done, record on log sheet as *Campylobacter* species. Repeat procedure for each sample blood agar plate. Swab the growth into Brucella broth with 15% glycerol mixture and freeze at -60 to -80°C. 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.

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; record results on the log sheet, 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; record results on the log sheet, 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; record results on the log sheet, 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; record results on the log sheet, select no for *Enterococcus*. If results produce catalase negative and PYR positive, record the isolate as positive for *Enterococcus*. Repeat procedure for each BHI agar plate. Sub culture one well isolated colony from BHI to blood agar plate. Incubate at 35°C for 24 hours.

Processing Day 5:

Salmonella

Examine each blood agar plate for purity. If pure, confirm as *Salmonella* by standard biochemical methods, API, VITEK or MALDI-TOF and serotype (optional). For sites that confirm and serotype both 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. Swab the growth into Brucella broth with 15% glycerol mixture and freeze at -60 to -80°C. 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 Processing 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; record results on the log sheet, 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. Laboratories should keep duplicates of strains within their culture collections.

Enterococcus

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

Whole Genome Sequencing:

*DO NOT SEQUENCE CHICKEN LIVERS, HEARTS OR GIZZARDS!

Refer to PulseNet and GenomeTrakr sequencing protocols. Also, refer to NARM's Sequencing Guidelines for additional instructions.

Preparing Isolates for Shipment:

Label each vial with NARMS isolate ID. The NARMS isolate ID on the vial should match the NARMS isolate ID on the log sheet. Labels **should not** be hand written or taped to the tube. Hand written or taped on labels come off during the freezing process. 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 (**DO NOT use excessive parafilm on the tubes**). 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 must 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 must 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:

Shawn McDermott
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