**Clinical and Molecular Determinants of ESBL Transmission in the Community**

**MERLIN Lab SOP**

**ESBL Confirmatory Testing**

**Materials & Equipment**

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| Ceftazidime/ceftazidime-clavulanic acid (ESBL CTZ/TZL Thermo Fisher Scientific 22-778-000/100pk))  Cefotaxime/cefotaxime-clavulanic Acid (ESBL CTX/CTL Thermo Fisher Scientific 22-777-997/100pk)  Cefepime/cefepime-clavulanic acid (RUO ESBL PM/PML Thermo Fisher Scientific 22-778-002/100pk)  Large Mueller Hinton (MH) agar plates (15 x 150mm) (Thermo Fisher Scientific R04050 10/pk or R04052 40/pk)  Buffered peptone water  Sterile saline (0.85% NaCl) (Thermo Fisher Scientific R064446 5ml/tube, 100/pk)  Sterile cotton swabs  Test tubes, pipettes and scissors  Sterile forceps  0.5 McFarland turbidity standard  Microbank tubes (Thermo Fisher Scientific, cat # 22-286-155)  Incubator (35 ± 2 °C)  Quality control organisms: *E. coli* ATCC 25922 (obtained from HUP Micro) and *K. pneumoniae* ATCC 700603 |

**Procedure:**

1. The research coordinator (RC) will review Theradoc for all new clinical Escherichia coli and Klebsiella spp from urine that are intermediate or resistant to ceftriaxone and/or ceftazidime. This corresponds to an MIC >2 for ceftriaxone and an MIC >4 for ceftazidime. In the event of a possible eligible subject, the RC will notify the lab technician who will then obtain the
2. Inoculum preparation. Emulsify several well-isolated colonies from an overnight agar plate in 3 ml saline to achieve a turbidity equivalent to a 0.5 McFarland standard. When the inoculum is correct, a confluent or almost confluent lawn of growth will be obtained after incubation. Perform regular colony counts to **verify that your procedure gives the correct inoculum density in terms of CFU/mL.**

Note: As the ESBL amount is inoculum dependent, too heavy or too light an inoculum may affect results. Excess enzyme may quench the clavulanic acid component in the test and potentially reduce the MIC ratio of CT/CTL or TZ/TZL and give a false negative result. On the contrary, too little enzyme may give a lower MIC for CT or TZ, and reduce the CT/CTL and TZ/TZL ratio.

1. Inoculation. Dip a sterile, swab into the inoculum suspension. Remove excess fluid by pressing the swab against the inside wall of the test tube. Swab the entire agar surface three times, rotating the plate approximately 60 degrees each time to ensure an even distri­bution of inoculum. Allow excess moisture to be absorbed for about 15 minutes so that the **surface is completely dry** before applying Etest ESBL strips.
2. Application. Check that the inoculated agar surface is **completely dry** before applying E-test ESBL strips. Open the package and handle the strips as described under **HANDLING**. E-test ESBL strips can be applied to the inoculated agar surface with a pair of forceps, a manual E-test applicator, or Nema C88. Always place the strip on the agar with the MIC scale facing upward i.e. towards the opening of the plate, and the antibiotic gradient on the agar surface. If incorrectly placed upside down, no ellipse will form because the antibiotic cannot diffuse across the non-porous plastic strip. Ensure the whole length of the strip is in complete contact with the agar surface. If necessary, remove air pockets by pressing gently on the strip with forceps, always moving from the lowest concentration upwards. Small bubbles under the strip will not affect results. Once applied, the strip cannot be moved as the antibiotic is instantaneously released into the agar.

5. Incubate the media overnight at 35 +/- 2°C.

6. The next day, read and record the MIC (where the edge of the inhibition ellipse intersects the strip) of each drug tested.

* 1. An ESBL test is positive if the MIC of the drug (CTX or CTZ) tested with clavulanic acid is 3, or more, two-fold dilutions greater than the MIC of the drug (CTX or CTZ) tested alone. It is also positive is a “phantom” zone or deformation of the CTX or CTZ is visible.
  2. An ESBL test may be “non-determinable” when bacterial growth is visible along the entire gradient strip. This is often caused by the organism producing an ESBL and/OR an AmpC beta-lactamase. A non-determinable test result can be confirmed as an ESBL producer using the cefepime/cefepime-clavulanic acid results.
  3. If an isolate is confirmed as producing an ESBL, catalog the sample in LabVantage (LV) and label and freeze the isolate in a Microbank Tube (Thermo Fisher Scientific, cat # 22-286-155)

7. Notify the RC that an ESBL was confirmed. The day of MIC determination starts the 7-day window within which the subject must be approached for enrollment.

**Post-enrollment**

Once a subject and their household has been enrolled, the lab can expect to receive swabs associated with the household three times over the course of the study (initial visit, one month and two months later).

Samples received should include:

* Two stool swabs from every household member
* Two fecal swabs from every dog/cat within the home
* Two Swiffer swabs of the nape of every dog/cat within the home. The Swiffer should be used to sample the neck and placed in a small labeled Whirl-Pak® bag
* Environmental swabs as follows:
  + Living room sampling (remote, TV, table)
  + Kitchen sampling (fridge handle, microwave handle, oven handle)
  + Index case bathroom sampling (sink basin, sink faucet, doorknob, toilet handle)
  + Communal bathroom sampling (sink basin, sink faucet, doorknob, toilet handle)
  + Washing machine sampling (inside, handle, doorknob)
  + Cell phone sampling (index case + each other person within the home)

**Procedures:**

Upon receipt of a households’ worth of swabs, check to make sure that all are present and follow the processing plan noted in the table below:

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| **Swab Type** | **Versions** | **Destination** |
| Stool (human)\* | Copan FLOQswab | Label, file & freeze |
| E-swab | Process & freeze |
| Feces (pet)\* | Copan FLOQswab | Label, file & freeze |
| E-swab | Process & freeze |
| Nape of neck (pet)\* | Swiffer/ Whirl-Pak® bag | Process |
| Copan FLOQswab | Label, file & freeze |
| Environmental (living room) | E-swab | Process & freeze |
| Copan FLOQswab | Label, file & freeze |
| Environmental (kitchen) | 3M sponge stick | Process, freeze residual |
| Copan FLOQswab | Label, file & freeze |
| Environmental (index bathroom) | 3M sponge stick | Process, freeze residual |
| Copan FLOQswab | Label, file & freeze |
| Environmental (communal bathroom) | 3M sponge stick | Process, freeze residual |
| Copan FLOQswab | Label, file & freeze |
| Environmental (washing machine) | 3M sponge stick | Process, freeze residual |
| Copan FLOQswab | Label, file & freeze |
| Environmental (cell phone/family member)\* | E-swab | Process & freeze |
| Copan FLOQswab | Label, file & freeze |

\*One set per household member/pet

**Stool (human) swab processing**

**Materials & Equipment**

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| * E-swabs to be processed | * 1mL sterile pipette |
| * ESBL Chromagar | * 1µL sterile loops | | * E-tests |
| * LabVantage labels | * 1.5mL cryovials | | * Mueller Hinton agar |
|  |  | |  |

1. Immediately label, file and freeze the Copan FLOQswab(s).
2. Create a child sample label from the E-swab for the plate.
3. Vortex the E-swab briefly. Obtain a drop’s worth (about 50µL) of E-swab fluid using 1mL sterile pipette and drop onto the ESBL chromagar. Use a 1µL loop to streak the plate for isolation. Label the plate(s) with the appropriate child sample LV label(s).
4. File and freeze the E-swab. Incubate the plate(s) at 35 +/-2°C overnight.
5. The next day, look at the ESBL Chromagar plates for presumptive ESBL colonies.
   1. *Escherichia coli* should produce pink colonies
   2. *Klebsiella pneumoniae* should produce blue colonies
6. If negative, no further action required.
7. If presumptive ESBL colonies are observed set up the 3 E-test strips on representative colonies using the procedure described above. Incubate the plates at 35 +/- 2°C overnight .
8. The next day read the MIC’s of the Etest strips and interpret based on the instructions above.

If the ESBL phenotype is confirmed create the appropriate child sample(s) of the plate and label the appropriate number of Microbank Tubes.

1. Inoculate a Microbank Tube with each confirmed ESBL isolate. File the tubes within LV and place into the -80°C freezer.
2. Sequester positive cultures for MALDI testing.

**Feces (pet) swab processing**

**Materials & Equipment**

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| * E-swabs to be processed | * 1mL sterile pipette | * Mueller Hinton agar |
| * ESBL Chromagar | * Sterile loops | * E-tests |
| * LabVantage labels | * 1.5mL cryovials |  |
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1. Immediately label, file and freeze the Copan FLOQswab(s).
2. Create a child sample label from the E-swab for the plate.
3. Use the E-swab to inoculate an ESBL Chromagar plate and use a 1ul to streak the plate. Label the plate(s) with the appropriate child sample LV label(s).
4. File and freeze the E-swab. Incubate the plate(s) at 35 +/-2°C overnight.
5. The next day, look at the ESBL Chromagar plates for presumptive ESBL colonies.
   1. *Escherichia coli* should produce pink colonies
   2. *Klebsiella pneumoniae* should produce blue colonies
6. If negative, no further action required.
7. If presumptive ESBL colonies are observed set up the 3 E-test strips on representative colonies using the procedure described above. Incubate the plates at 35 +/- 2°C overnight.
8. The next day read the MIC’s of the E-test strips and interpret based on the instructions above.
   1. If the ESBL phenotype is confirmed create the appropriate child sample(s) of the plate and label the appropriate number of Microbank Tubes.
9. Inoculate a Microbank Tube with each confirmed ESBL isolate. File the tubes in LV and place into the -80°C freezer.
10. Sequester positive cultures for MALDI testing.

**Nape of Neck (pet) swab processing**

**Materials & Equipment**

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| * Swiffer Cloths | * 1mL sterile pipette | * E-tests |
| * ESBL Chromagar | * Sterile loops |  |
| * LabVantage labels | * Whirl-Pak Bags | * Mueller Hinton agar |
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1. Add 100 ml of 1 X Buffered Peptone Water to the Whirl-Pak bag and incubate the Swiffer cloth overnight at 35 +/- 2°C overnight.
2. Using a 10 ul loop to streak an ESBL Chromagar plate and label the plate(s) with the appropriate LV label(s).
3. Incubate the plate(s) at 35 +/- 2°C overnight.
4. The next day, look at the ESBL Chromagar plates for presumptive ESBL colonies.
   1. *Escherichia coli* should produce pink colonies
   2. *Klebsiella pneumoniae* should produce blue colonies
5. If negative, no further action required.
6. If presumptive ESBL colonies are observed set up the 3 E-test strips on representative colonies using the procedure described above. Incubate the plates at 35 +/- 2°C overnight.
7. The next day read the MIC’s of the E-test strips and interpret based on the instructions above.
   1. If the ESBL phenotype is confirmed create the appropriate child sample(s) of the plate and label the appropriate number of Microbank Tubes.
8. Inoculate a Microbank Tube with each confirmed ESBL isolate. File the tubes within LV and place into the -80°C freezer.
9. Sequester positive cultures for MALDI testing.

**Environmental swab processing**

**Materials & Equipment**

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| * Sterile PBST (0.02%) | * Sterile 10µL & 1000µL tips | * E-tests |
| * Sterile 50mL tubes * Sterile 1.5mL cryovials * Sterile 5mL cryovials * Sterile 5mL and 50mL pipettes * 10µL and 1000µL pipettors | * Sterile loops * Stomacher bag rack * Stomacher 400C circulator * Table top centrifuge * Multi-tube vortex mixer * LabVantage labels | * Mueller Hinton agar |
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* + - 1. Place sample bags with environmental sponges into a stomacher bag rack and add 45mL of PBST to each bag with a sterile 50mL serological pipette.
      2. Remove plastic backing and open the sponge so that it is not doubled over onto itself. Aseptically orient the long side of the sponge with the bottom of the bag. Squeeze the sponge until it is fully saturated in PBST.
      3. Process each swab within the stomacher 400C circulator for 1 minute at 200 RPM. Allow foam to reduce in homogenate for 5-10 minutes.
      4. Carefully hold and squeeze the sponge on one side of the bag and from the opposite side remove all of the homogenate from the stomacher bag into a labeled sterile 50 mL centrifuge tube using a sterile 50mL pipette.
      5. Concentrate homogenates by centrifuging at 2000 x g for 30 minutes.
      6. Remove the supernatant from the tube using a 50mL pipette, leaving 5mL of supernatant in the tube as indicated by the graduated markings on the tube.
      7. Place tube(s) on VWR multi-tube vortexer for a total of 2 minutes with 10 second bursts.
      8. Vortex again for a few seconds and then measure the final volume (~5mL).
      9. Plate 50µl for isolation from undiluted suspension onto selective ESBL agar. Each plate should be labeled with a LabVantage label.
      10. The remaining ~5mL can be stored in a labeled 5mL cryovial. This can then be placed at -80C.

1. Incubate the plates at 35 +/-2°C overnight.
2. The next day, look at the ESBL Chromagar plates for presumptive ESBL colonies.
   1. *Escherichia coli* should produce pink colonies
   2. *Klebsiella pneumoniae* should produce blue colonies
3. If negative, no further action required.
4. If presumptive ESBL colonies are observed set up the 3 E-test strips on representative colonies using the procedure described above. Incubate the plates at 35 +/- 2°C overnight.
5. The next day read the MIC’s of the E-test strips and interpret based on the instructions above.
   1. If the ESBL phenotype is confirmed create the appropriate child sample(s) of the plate and label the appropriate number of Microbank Tubes.
6. Inoculate a Microbank Tube with each confirmed ESBL isolate. File the tubes in LV and place into the -80°C freezer.
7. Sequester positive cultures for MALDI testing.

**MALDI Procedure**

**Materials & Equipment**

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| * Sterile toothpicks |
| * Matrix solution * Microscope slides * *E. coli* control   \*\* each MALDI slide has 45 spots |

1. Using a sterile toothpick, select one colony from the plate of interest and carefully spread it onto a new spot on the MALDI slide.
2. Drop 1µL of matrix solution over the spot and allow to air dry completely.
3. Record the ID of the colony on the MALDI slide.
4. Repeat steps 1-3 for any plates requiring MALDI identification.
5. Use *E. coli* control for the center spot.
6. Document the MALDI slide in BOX.
7. Deliver the set of slides to HUP Micro (4 Gates) before the end of the day.
8. Upon receipt of results, record them in the BOX file.

**Solution Recipes**

1L 0.02% PBST

* 200 µl Tween
* 100 mL 10X PBS
* 900 mL dH20

Peptone Water

* 7.5g medium
* 500mL demineralized water
* Autoclave