CHN61: EXTENDED-SPECTRUM BETA-LACTAMASE (ESBL) TESTING

1.1 Introduction

A common mechanism of bacterial resistance to beta-lactam antibiotics is the production of beta-lactamase enzymes that break down the structural beta-lactam ring of penicillin and penicillin-like drugs, such as Cephalosporins.

Extended-spectrum beta-lactamases (ESBLs) are enzymes produced in some gram-negative bacilli that mediate resistance to extended-spectrum Cephalosporins and aztreonam.

ESBLs are most commonly recognized in Escherichia coli, but have been detected in a variety of Enterobacteriaceae and Pseudomonas aeruginosa isolates as well. Of particular regional interest is the detection of ESBLs in Salmonella spp, Shigella spp and Escherichia coli.

Enterobacteriaceae that have a zone of inhibition of 27mm and below for Cefotaxime, a zone of inhibition of 22mm and below for Ceftazidime and are resistant or intermediate to Ceftriaxone should be tested for the production of ESBL's. Until such testing is completed, the results of disk diffusion susceptibility tests for Ampicillin, Augmentin, or any Cephalosporin should not be reported. When production of ESBL is confirmed then the isolate must be reported as resistant to Ampicillin, Augmentin, and all Cephalosporins regardless of the individual disk diffusion result.

1.2 Purpose

This SOP provides guidance on how to test *Salmonell species*, Escherichia coli and Shigella species that have a zone of inhibition of 27mm and below for Cefotaxime, and a zone size of inhibition of 22mm and below for Ceftazidime or are resistant or intermediate to Ceftriaxone for the production of ESBL's.

2.0 Responsibility

This SOP is applicable to laboratory staff working in the CHAIN Study in Microbiology laboratory sections.

The Principal Investigator (through the study coordinator when applicable) retains the overall responsibility of implementation of these standard procedures.

The Study Laboratory Coordinator is responsible for answering questions you may have about the content of this SOP and any other relevant study documentation. Please contact that the Study Laboratory Coordinator through your site coordinator.

3.0 Safety/Risk Assessment

3.1 Personal protective equipment such as gloves and laboratory coats must be worn at all times while handling microorganisms.

3.2 Observe laboratory precautions at all times.

3.3 Care should be taken while handling suspensions to avoid generation of aerosols.

3.4 The biosafety cabinet should be used when handling suspected *Salmonella* species and, *Shigella* species.

4.0 Abbreviations/Definitions

ESBLs	Extended-spectrum beta-lactamases
CTX	Cefotaxime
CAZ	Ceftazidime
NCTC	National Culture Type Collection (UK)
ATCC	American Type Culture Collection (USA)
CTX/CLA	Cefotaxime-clavulanate
CAZ/CLA	Ceftazidime-clavulanate

5.0 Specimen

Escherichia coli, Salmonella and *Shigella* species that have a zone of inhibition of 27mm and below for Cefotaxime, and a zone size of inhibition of 22mm and below for Ceftazidime or are resistant or intermediate to Ceftriaxone.

6.0 Equipment / Materials/ Reagents

6.1 Equipment

Aerobic Incubator at 37^0C

Vernier caliper

6.2 Required material

Mueller Hinton agar (MHA).
Antibiotic discs (CTX 30, CAZ30, CTX-clavulanate 30-10, CAZ clavulanate 30-10)
E. coli (ECO) ATCC 25922 negative Control.
E. coli (ECO) NCTC 13351 positive control
0.5 McFarland standard

6.3 Reagents

Sterile normal saline

7.0 Methods

7.1 Prepare a bacterial suspension of test organism equivalent to 0.5 McFarland standards in sterile normal saline.

7.2 Dip a sterile cotton wool swab into the suspension and remove excess liquid by turning the swab against the side of the container

7.3 Spread the inoculum evenly over the entire surface of the plate by swabbing in three directions. Allow the plate to dry for no more than 15 minutes before applying the discs

7.4 Positive control TEM-3 (broad-spectrum) *E. coli* NCTC 13351 and negative (*E. coli* ATCC 25922) control strains are inoculated onto separate plates when there is failure in the weekly AST testing QC.

7.5 A Ceftazidime 30mg disc, a Ceftazidime-Clavulanate disc, a Cefotaxime 30mg disc and a Cefotaxime-Clavulanate discs are then placed onto an MHA plate.

7.6 Incubate the plates at 37° C for 18-20 hours.

7.7 Measure the zone sizes around each disc. ESBL production is inferred when the zone of inhibition around the Ceftazidime-Clavulanate or Cefotaxime-Clavulanate discs is expanded by >5mm compared to the respective Ceftazidime or Cefotaxime discs alone.

8.0 Reporting of Results.

Clinical isolates of *E.coli*, *Shigella species* and *Salmonella* found to produce ESBLs should be assumed to be resistant to all penicillins and cephalosporins (except cefoxitin) irrespective of susceptibility testing.
If the positive control is not found to have an ESBL, or if the negative control is found to have an ESBL, then the test is invalid and must be repeated.

9.0 Appendices:

None

10.0 References:

1. BSAC Methods for Antimicrobial Susceptibility Testing. Version 9, January 2010 British Society for Antimicrobial Chemotherapy.
2. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement.*CLSI document M100-S24. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
3. Sanders CC, Sanders WE Jr. ß-lactam resistance in gram-negative bacteria: global trends and clinical impact. Clin Infect Dis 1992; 15:824-83
4. BSAC. Detection of extended-spectrum beta-lactamases (ESBLs) in E. coli and Klebsiella species. www.bsac.org

5. Livermore DM. ß-lactamases in Laboratory and Clinical Resistance. Clin Microbiol Rev 1995; 8:557-584
6. Livermore DM. Detection of beta-lactamase mediated resistance. UK Health Protection Agency. www.bsac.org
7. Health Protection Agency (xxxx). Laboratory detection and reporting of bacteria with extended spectrum beta-lactamases. National Standard Method QSOP 51 Issue x. http://www.hpa-standardmethods.org.uk/pdf_sops.asp.

11.0 Document history

Version	Author	Approved by	Dated
1.01 **CHAIN BLOOD SPOT COLLECTION SOP (MASTER) CHN 28**	Robert Musyimi	Caroline Tigoi	10/10/2016
1.02			

12.0 Site training record

All sites are required to maintain a master copy of this SOP that documents the site staff that have been trained on this SOP.

Document History				
Version No.	**Trained staff initials**	**Signature of trained staff**	**Date**	**Trainer's Initials**
1.01	KDT	Example row	1st Jan 2016	DM