

CHAIN PROCESSING OF RECTAL SWABS FOR CARRIAGE OF ESBL POSITIVE



ENTEROBACTERIACEAE SOP (MASTER)

CHN60: PROCESSING OF RECTAL SWABS FOR CARRIAGE OF ESBL POSITIVE ENTEROBACTERIACEAE.

1.0 PURPOSE / INTRODUCTION:

Introduction:

Normal flora, which is important for the maintenance of health, can also act as a reservoir for transmission of bacteria that may cause infectious diseases. Acquisition of carriage of bacteria such as MRSA, ESBL-positive *Enterobacteriaceae* and penicillin-resistant pneumococci may result in infections with antibiotic-resistant bacteria. Screening patients for carriage of these bacteria can help manage and control spread of resistant strains.

Purpose:

To give guidance on the microbiological processing of rectal swabs for detection of ESBL-positive *Enterobacteriaceae*.

2.0 SCOPE / RESPONSIBILITY:

This SOP is applicable to all lab staff working in microbiology.

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 Observe standard laboratory precautions at all times.
- 3.2 Personal protective equipment such as gloves and laboratory coats must be worn at all times while handling microorganisms.
- 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 Typhi* and any other highly pathogenic organism.

4.0 DEFINITIONS:

- 4.1 GENT: MAC: MacConkey agar 8% Gentamicin
- 4.2 ESBL: Extended Spectrum Beta Lactamase
- 4.3 SOP: Standard Operating Procedures
- 4.4 BAP: Blood agar plate
- 4.5 KIDMS: Kilifi Integrated Data Management System

5.0 SPECIMEN:

Freshly collected rectal swabs.

6.0 EQUIPMENT / MATERIALS/ REAGENTS:

6.1 Equipment:

- 6.1.1 Aerobic (O₂) incubator
- 6.1.2 Aerobic (5% CO₂) incubator
- 6.1.3 Fridge

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- 6.1.4 Forceps
- 6.1.5 Antibiotic disc dispenser

6.2 Materials:

- 6.2.1 - Media plates (Gent-Mac, BA, MHA)
- 6.2.2 - Nichrome wire loop
- 6.2.3 - Sterile cotton tipped swabs
- 6.2.4 - Bunsen burner
- 6.2.5 Ceftriaxone and Ceftazidime discs

7.0 METHODOLOGY:

7.1 Procedure:

7.1.1 Day one:

- 7.1.1.1 Register the request form information into the online computer database (LIMS).
- 7.1.1.2 If a positive result has been entered for a previous swab from the same neonate, comment that an 'ESBL positive isolate has been isolated from the previous swab' on the specimen page in the online database and on the sample request form.

7.1.1.3 Media plates:

- 7.1.1.3.1 Obtain media plates that have passed QC for culture from the media fridge in the media room and place them on the working bench. The concentration and volume of Gentamicin to be added to MacConkey media to make 8% GENT-MAC agar is shown in the appendix 8.1.
- 7.1.1.3.2 Leave the culture plates on the working bench in order to attain room temperature.
- 7.1.1.3.3 Label the media plates for culture with the patient admission serial number, date of processing and sample type.

7.1.2 Culture:

- 7.1.2.1 Pull out the swab from the transport medium, ready for inoculation.
- 7.1.2.2 Inoculate the surface of the media plates by rolling the swab onto Blood Agar Plate (BAP) first and then to the Gent-MacConkey Agar (GENT-MAC). This order should be adhered to at all time since BA is an enriched media and GENT-MAC is a selective media.
- 7.1.2.3 Streak the inoculum you have just made using a pre-flamed and cooled wire loop, and incubate the GENT-MAC at 37°C in the aerobic incubator and BAP in the 5% CO₂ incubator.
- 7.1.2.4 For the BAP place a Ceftriaxone (CRO) disk (30ug), on streak 2 and a Ceftazidime (CAZ) disc on streak 4 (Appendix 8.2). This will act as a supplementary test for Ceftriaxone and Cetazidime resistance.

7.1.3 Day two:

- 7.1.3.1 Reporting of cultures
 - 7.1.3.1.1 Examine the plates for any growth obtained.
 - 7.1.3.1.2 For the BAP plates, look at growth on the second and fourth streak, any colonies within 25mm diameter of the CRO disc and 22mm diameter on the CAZ disc should be plated out for identification. For the GENT-MAC plate, all colony types should be examined further.

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7.1.3.1.3 Gram stain the different colonies to ascertain that they are gram-negative bacilli. Perform an oxidase test on the gram-negative rods, and if negative, this is likely to be gram-negative enteric bacteria.

7.1.3.1.4 Plate out the different gram-negative colonies onto a purity plate (Blood Agar) for identification. Label the colony-types on your worksheet and on the plates.

7.1.3.1.5 Re-incubate the plate(s) for further 18-24 hours if **NO GROWTH** is obtained.

7.1.3.1.6 Biochemical testing should be done to identify the bacteria of differing colony morphologies that were identified as potentially Gentamicin resistant from the GENT-MAC media and Ceftriaxone and Ceftazidime resistant gram-negative rods (GNR) from the BAP.

7.1.4 Day three:

7.1.4.1 Reporting of cultures, identification and antimicrobial susceptibility testing

7.1.4.1.1 Examine the plates and identify any growth obtained systematically referring to specific SOPs for the different enteric organisms.

7.1.4.1.2 If growth is observed in the re-incubated plates, follow procedure as from step 7.1.3.1.2

7.1.4.1.3 Biochemical tests are done to identify the bacteria from the different colony morphologies (for the re-incubated plates.)

7.1.4.1.4 Set up antibiotic susceptibility testing) and ESBL testing (ESBL testing SOP) on the isolates identified as *Enterobacteriaceae*.

7.1.4.1.5 All the results from the processing should be entered into the request form as well as in the LMIS database.

7.1.4.1.6 All isolates identified as *Enterobacteriaceae* and are ESBL positive should be frozen in an ultra-low freezer -80°C (Freezing SOP)

7.1.5 Quality Control:

7.1.5.1 Weekly quality control assays will be done using control organisms to test for ESBL production. Results of such assays should be according to the accepted range provided by the CLSI guidelines so as to validate the results generated.

7.1.5.2 Deviation of such results to the accepted range will result in quality control assays to be done daily until these results are according to the accepted range where weekly quality control assays will be done.

7.1.5.3 Quality control for the GENT-MAC media should be done on every batch of media prepared. Use the designated positive control organism e.g. *Klebsiella pneumoniae* and the negative control organism e.g. *E. coli* available in your laboratory

8.1 Calculation of concentration of Gentamicin introduced into the MacConkey Agar to make Gentamicin MacConkey Agar.

Concentration to attain 8mg/L

Manufacturer's concentration= 10mg/ml

10mg/ml= 10,000mg/L

Therefore Aliquot volume from Manufacturer's concentration to get 8mg/L is;

Concentration 1 X Volume 1= Concentration 2 X Volume 2

10,000mg/L X Volume 1= 8mg/L X 1000mL

Volume 1= 8000/ 10,000

Volume 1= 0.8ml

Volume1 = 800ul

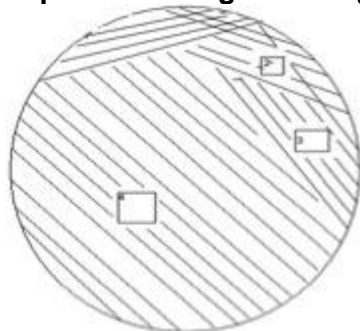
Therefore for one to get a concentration of 8mg/L, one needs to aliquot 800ul of gentamicin (from the Manufacturer's concentration) per 1L of MacConkey Agar.

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8.2 Example plate showing streaking method, 2nd, 3rd and 4th streak.



9.0 REFERENCES:

1. Clinical laboratory standards Institute (CLSI) Document- Performance Standards for Antimicrobial Susceptibility Testing M100-S23 2013 12th Edition Vol. 33 No. 1
2. Baba H, Nimmo GR, Allworth AM, Boots RJ, Hayashi Y, Lipman J, Paterson DL: The role of surveillance cultures in the prediction of susceptibility patterns of Gram-negative bacilli in the intensive care unit. Eur J Clin Microbiol Infect Dis 2011, 30:739-744.

Document history

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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 | | | | |
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