APPENDIX II – DOUBLE DISK DIFFUSION for ERYTHROMYCIN and CLINDAMYCIN on β-haemolytic Streptococci Groups A, B, C, G and Streptococcus pneumoniae

I. Introduction

Macrolide (erythromycin) resistant β-haemolytic Streptococci and Streptococcus pneumoniae isolates may have constitutive or inducible resistance to lincosamides (clindamycin). The mechanisms of resistance include:

- Ribosomal modification encoded by an \textit{erm} gene; also refer to as MLS\textsubscript{B} (macrolide, lincosamide and type B streptogramin) resistance.
- Efflux of the antibiotic encoded by a \textit{mef} gene; resistant only to macrolide
- Drug inactivation

Inducible clindamycin resistance can be detected using a disk approximation test with a clindamycin disk placed beside an erythromycin disk as part of the normal disk diffusion test.

II. Materials

Antimicrobial disks – clindamycin (DA, 2 µg) and erythromycin (E, 15 µg)
Mueller Hinton Blood Agar (MHB)
Trypticase Soy Broth (TSB) (3 mL)
VITEK colorimeter
Sterile saline
Sterile swabs

III. Procedure

1. Allow disks to come to room temperature before opening the container.

2. Using the Vitek colorimeter, prepare a suspension of the test organism in sterile saline equivalent to a 0.5 McFarland standard using isolated colonies.

3. Using a sterile cotton swab, inoculate the standardized organism onto an MHB agar plate, streak in three directions over the entire agar surface.

4. Place plate on disk template (Figure 1.)

5. Using forceps or a disk dispenser, apply the clindamycin and erythromycin disks onto the agar 12 mm apart from each other edge to edge using template below (Figure 1). Other antimicrobial disks can be placed on the same agar plate if needed.
6. Incubate plates in CO₂ at 35°C for 20 to 24 hours

IV. Interpretation

1. After incubation, measure the diameters of the zone of complete inhibition with callipers/ruler. Refer to Clinical and Laboratory Standards Institute (CLSI) Document - M100-S15 (M2) for the zone size interpretations.
2. Enter zone size measurements into the LIS.
3. Organisms that show flattening of the clindamycin zone adjacent to the erythromycin disk in the shape of the letter D (referred to as a “D” zone) have inducible clindamycin resistance. Enter into the LIS the presence or absence of “D” zone as “Y” or “N” under LIS drug “D zone”. Isolates that show the presence of D zone will be automatically reflexed in the LIS to report as “clindamycin resistant”.

Figure 1. Template for Clindamycin and Erythromycin disks placement
Examples of Zone of Inhibition Patterns and their Interpretation

Both E and DA are Susceptible.
Report both E and DA as S

Both E and DA (measured at the narrowest side) are I or R; “D” zone is positive – Inducible MLSβ; presumed genotype: erm
Report both E and DA as R

Both E and DA I or R – Inducible or constitutive MLSβ; presumed genotype: erm
Report both E and DA as R

E is I or R and DA is S – M phenotype; presumed genotype: mef.
Report E as I or R and DA as S.
V. Quality Control

See Clinical and Laboratory Standards Institute (CLSI) Document - M100-S15 (M2) Table 3 for acceptable QC results.

VI. References


Streptococci and Staphylococcus (overview of macrolides and lincosamide resistance)
Leclercq. CID 2002;34:482-92

Streptococcus pneumoniae
Descheemaeker et al. JAC 2000 45:167-173

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GAS Descheemaeker et al. JAC 2000 45:167-173
GBS de Azavedo et al. AAC 1001;45:3504-3508
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