ANNEXURE
IX. ANNEXURE

REAGENTS FOR β-LACTAMASE TESTS\[155\]

A) Iodometric cell suspension method:

1. Starch solution (1%) in distilled water, boiled to dissolve (prepared fresh each day)
2. Iodine reagent:
   - Iodine - 2.03 gm
   - Potassium iodide - 53.2 gm
   - Distilled water - 100 ml.
3. Penicillin G 10,000 units (6.06 mg) in 0.1M Phosphate buffer p\text{H} 7.
4. Phosphate buffer p\text{H} 7
   Prepare stock solution A and B

a) Ingredients
   i. Stock phosphate solution A
      - Sodium di-hydrogen phosphate - 27.69 gm.
   ii. Stock phosphate solution B
      - Distilled water - 100 ml.

b) Preparation of phosphate buffer p\text{H} 8
   - Stock solution A - 5.3 ml
   - Stock solution B - 9.7 ml
   - Distilled water - 100 ml.

Preparation of phosphate buffer p\text{H} 7
   - Stock solution A - 39 ml
   - Stock solution B - 61 ml
   - Distilled water - 100 ml.

ANTIBIOTIC SUSCEPTIBILITY TESTS\[65,130,168,169,173,174,175\]

Minimum Inhibitory Concentration (MIC):

Antimicrobial agents:

Preparation of benzyl penicillin G, ciprofloxacin, tetracycline, gentamicin, streptomycin, vancomycin and teicoplanin for known potency suitable for laboratory use obtained from Hi-Media laboratory, Mumbai. Suitable diluents and storage conditions are
given in following table. Containers with compounds held at -20\(^{0}\) C allowed to come to room
temperature before opening to avoid condensation of water.

**Preparation and Storage of Antibiotic Solutions**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Antimicrobial agents</th>
<th>Diluents</th>
<th>Storage of solution</th>
<th>Storage of powder*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Penicillin G</td>
<td>Distilled water</td>
<td>---</td>
<td>1 month</td>
</tr>
<tr>
<td>2.</td>
<td>Ciprofloxacin</td>
<td>Distilled water</td>
<td>2 weeks</td>
<td>3 months</td>
</tr>
<tr>
<td>3.</td>
<td>Tetracycline</td>
<td>Distilled water</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>4.</td>
<td>Gentamicin</td>
<td>Distilled water</td>
<td>6 months</td>
<td>---</td>
</tr>
<tr>
<td>5.</td>
<td>Streptomycin</td>
<td>Distilled water</td>
<td>6 months</td>
<td>---</td>
</tr>
<tr>
<td>6.</td>
<td>Vancomycin</td>
<td>Distilled water</td>
<td>1 week</td>
<td>3 months</td>
</tr>
<tr>
<td>7.</td>
<td>Teicoplanin</td>
<td>Distilled water</td>
<td>1 week</td>
<td>3 months</td>
</tr>
</tbody>
</table>

*= protected from light & moisture, RT = Room temperature

**Stock solutions of antibiotics and preparation of media**

It is conventional to prepare dilutions of antibiotic based on \(\mu\) g/ml i.e. doubling
dilutions method. The different concentration ranges used to determine the MIC testing are
given below.

The weight of antibiotic in \(\mu\)g/ml required calculated by the following formula:

\[
W = \frac{1000}{P} \times V \times C
\]

Where,

- \(W\) = Weight (\(\mu\)g) of antibiotic to be dissolved in \(V\),
- \(P\) = Potency of preparation in relation to base,
- \(V\) = Volume (ml) required,
- \(C\) = Final concentration of solution.

For e.g. benzyl penicillin G

\[
W = \frac{1000}{990} \times 25 \times 256
\]

\(P = 990 \, \mu\)g/ mg,

\(V = 25\) ml (for single plate)

\(W = 6464.64 \, \mu\)g/ ml \(C = 256 \, \mu\)g/ ml (double than highest concentration)

\(= 6.46 \, \text{mg/ ml}\)
= 12.92 mg/ 2 ml  
0.5 ml* (3.23 mg)  (3230 μg/ 0.5ml)  
(1) 0.5 ml* (3230 μg/ 0.5ml)  
+ 24.5 Molten MHA agar  
25 ml (3230 μg/ 25ml)  
(3230/25=129.2 μg/ ml)  

P= 990 μg/mg (990 μg/ 1000 μg)  
\[
\frac{C}{1000} = \frac{990}{1000} \times 129.2 \quad C = 127.9
\]

C = 128 μg/ ml (highest concentration used in MIC)  
(2) 0.5 ml* (3230 μg/ 0.5ml)  
+ 0.5 ml (distilled water)  + 24.5 Molten MHA agar  
1 ml (3230 μg/ ml) 25 ml (3230 μg/ 25ml)  
C = 64 μg/ ml.  
(3) 0.5 ml (1615 μg/ 0.5ml)  
+ 0.5 ml (distilled water)  + 24.5 Molten MHA agar  
1 ml (1615 μg/ ml) 25 ml (807.5 μg/ 25ml)  
C = 32 μg/ ml.  
(4) 0.5 ml (807.5 μg/ 0.5ml)  
+ 0.5 ml (distilled water)  + 24.5 Molten MHA agar  
1 ml (807.5 μg/ ml) 25 ml (403.75 μg/ 25ml)  
C = 16 μg/ ml.  

And so on  
(8) C = 1 μg/ ml.  (9) C = 0.5 μg/ ml.  (10) C = 0.25 μg/ ml.
1) Combined disc method

The weight of antibiotic in µg/ml required was calculated by the following formula:

10 mg streptomycin dissolved in 4.75 ml of distilled water.

\[
\frac{10,000 \, \mu g}{4.75 \, ml} = \frac{1052 \, \mu g}{0.5 \, ml} + 0.5 \, ml \text{ (distilled water)}
\]

\[
\frac{1052 \, \mu g}{1 \, ml} = \frac{1052 \, \mu g}{1000 \, \mu l}
\]

\[
\frac{10.52 \, \mu g}{10 \, \mu l} = \frac{9.99 \, \mu g}{10.52}
\]

Potency is 950 / mg \((950/1000 \, \mu g)\)

\[
950/1000=?/10.52
\]

\[
? = 950 \times 10.52/1000
\]

\[
?=9.99 \, \mu g
\]

10.52 µg streptomycin powder contains exact 10 µg streptomycin antibiotic.

Preparation of combined discs:

Readymade disc of penicillin (10 Units/disc) purchased from Hi-Media, Mumbai were used for combined disc synergy test.

1. Penicillin G (10 Units/disc) discs were arranged separately in sterile Petri-dishes.
2. With the help of sterile micro-pipette 10 µl distilled water containing 10.52 µg\(^{(*)}\) antibiotic were delivered on single disc and allowed to dry in the incubator at 37\(^{0}\) C for 30 minutes.
3. The discs were preserved in sterile screw caped bottle containing silica gel.
4. If these discs were not used on the day of preparation and stored at -2\(^{0}\) C to +8\(^{0}\) C and then used for Kirby-Bauer disc diffusion method.
PROFORMA

A. Patient’s history

Sr. No. : Date:
Name : Age / Sex:
Name of hospital: IPD/OPD No.
Name of Lab : Lab. No.
Clinical history : Specimen:

B. Culture & Identification

Blood agar
MacConkey’s agar
Gram stain Motility

Biochemical tests:

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase</td>
<td>Salt tolerance test</td>
<td>Growth on PT</td>
<td></td>
</tr>
<tr>
<td>Bile aesculin</td>
<td>pH tolerance test</td>
<td>Growth at 45°C</td>
<td></td>
</tr>
<tr>
<td>PYRase</td>
<td>Arginine hydrolysis</td>
<td>Penicillinase</td>
<td></td>
</tr>
</tbody>
</table>

Sugar fermentation tests:

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Arabinose</th>
<th>Lactose</th>
<th>Xylose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mannitol</td>
<td>Raffinose</td>
<td>Inulin</td>
<td></td>
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<tr>
<td>Sorbitol</td>
<td>Sucrose</td>
<td>Pyruvate</td>
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</table>

Name of species: Vitek 2 identification

Group/Biotype Associate Species:
C. Antibiotic sensitivity testing

**Disc diffusion Method**

<table>
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</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td></td>
<td>Ampicillin</td>
<td></td>
<td>Rifampin</td>
<td></td>
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<tr>
<td>Amoxycillin</td>
<td></td>
<td>Ampi + Sulb</td>
<td></td>
<td>Linezolid</td>
<td></td>
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<tr>
<td>Amoxyclave</td>
<td></td>
<td>Piperacillin</td>
<td></td>
<td>Pristinomycin</td>
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<tr>
<td>Imipenem</td>
<td></td>
<td>Pippertazobact</td>
<td></td>
<td>Vancomycin</td>
<td></td>
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<tr>
<td>Tetracycline</td>
<td></td>
<td>Doxycycline</td>
<td></td>
<td>Teicoplanin</td>
<td></td>
</tr>
<tr>
<td>Azithromycin</td>
<td></td>
<td>Erythromycin</td>
<td></td>
<td>HL Gentamicin</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td>Gatifloxacin</td>
<td></td>
<td>HL Streptomycin</td>
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<tr>
<td>Nitrofurantoin</td>
<td></td>
<td>Fosfomycin</td>
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</table>

**Dilution Method**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Peni</th>
<th>Tetra</th>
<th>Cipro</th>
<th>Vanco</th>
<th>Teico</th>
<th>HLGR</th>
<th>HLSR</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIC (µg/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>500</td>
<td>2000</td>
</tr>
<tr>
<td>S/R</td>
<td></td>
<td></td>
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</tbody>
</table>

**Antibiotic synergism**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Combined disc Diffusion (S/R)</th>
<th>Time kill curve (S/R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin + Gentamicin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillin + Streptomycin</td>
<td></td>
<td></td>
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</tbody>
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