Detection of Extended-Spectrum β-Lactamases (ESBL) in *Klebsiella pneumoniae, Klebsiella oxytoca, Escherichia coli* and *Proteus mirabilis*

Purpose

To evaluate the β-Lactamases enzyme in *K. pneumoniae*, *K. oxytoca*, *E. coli*, and *P. mirabilis*

Materials

1. Overnight culture of bacterial isolate on non-selective media (Tryptic soy agar (TSA), Sheep blood agar (SBA))
2. Muller Hinton Agar (MHA)
3. Muller Hinton Broth (MHB)
4. Nephelometer
5. 0.5 McFarland standard
6. Sterile cotton swab
7. Sterile forceps
8. 35°C ambient-air incubator
9. Antimicrobial agents
    - **Disk diffusion**
      Cefotaxime 30 µg
      Cefotaxime-clavulanate 30/10 µg
      Ceftazidime 30 µg
      Ceftazidime-clavulanate 30/10 µg
10. Quality control strains
    - When performing the ESBL test, *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 should be used for routine QC (e.g., weekly or daily).
        - *K. pneumoniae* ATCC 700603
            - Disk diffusion: ≥ 5-mm increase in zone diameter of ceftazidime-clavulanate vs ceftazidime alone
            - Disk diffusion: ≥ 3-mm increase in zone diameter of cefotaxime-clavulanate vs cefotaxime alone
        - *E. coli* ATCC 25922
            - Disk diffusion: ≤ 2-mm increase in zone diameter for antimicrobial agent tested in combination with clavulanate vs the zone diameter when tested alone.

Procedure

1. Make a direct broth suspension of isolated colonies selected from an 18- to 24-hrs non-selective agar plate.
2. Adjust the turbidity of culture with MHB to achieve a turbidity equivalent to a 0.5 McFarland standard. **Use the inoculum within 15 mins.**
3. Dip a sterile cotton swab into the 0.5 McFarland adjusted suspension and rotate the swab several times and press firmly on the inside wall of the tube above the fluid level.
4. Inoculate the dried surface of an MHA plate by streaking the swab over the entire sterile agar surface.
5. Repeat this procedure by streaking 2 more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum.
6. Place antibiotic disk (Cefotaxime, Cefotaxime-clavulanate, Ceftazidime, Ceftazidime-clavulanate) on the surface of the inoculated plate.
7. Press each disk down to ensure complete contact with the agar surface.
8. Invert the plates and place them in an incubator set to 35°C±2°C within 15 mins after the disks are applied for 16-18 hrs.
9. Measure the zone diameter (mm.) of the antibiotic disk.

Interpretation of results

Disk diffusion

- ≥5 mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanate vs the zone diameter of the agent when tested alone = ESBL (e.g., ceftazidime zone = 16 mm; ceftazidime-clavulanate zone = 21 mm).

References

CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 32nd ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2022.

CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 13th ed. CLSI supplement M02. Clinical and Laboratory Standards Institute; 2018.