Kirby-Bauer Disk Diffusion Susceptibility Test Protocol

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Information

History

The publication on penicillin by Alexander Fleming in 1928 is a milestone in the history of medicine. As more antimicrobial compounds were discovered, it was predicted that infectious diseases would be eliminated through the use of these antimicrobials (7). Unfortunately, the development of bacterial resistance to these antimicrobials quickly diminished this optimism and resulted in the need for physicians to

susceptibility test, henceforth called the Kirby-Bauer disk diffusion test (2).

Currently, the Clinical Laboratory Standards Institute (CLSI) is responsible for updating and modifying the original procedure of Kirby and Bauer through a global consensus process. This ensures uniformity of technique and reproducibility of results as pathogens develop new mechanisms of resistance and new antimicrobials are developed to fight these organisms. Interpretative guidelines for zone sizes are included in their publications (3). The CLSI publication, Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard 9th Edition, represents the standard for clinical laboratories performing susceptibility testing today.

Purpose

The purpose of the Kirby-Bauer disk diffusion susceptibility test is to determine the sensitivity or resistance of pathogenic aeroy216 52Tw 9.4eo9.96 -0 0 9.9

Mueller-Hinton agar_

MH agar is considered the best medium to use for routine susceptibility testing of nonfastidious bacteria for the following reasons:

inhibition. Conversely, plates poured to a depth >4 mm will result in false resistant results.

· pH of the MH agar should fall between 7.2 and 7.4 at room temperature after solidification and should be tested when the media is drugs will appear to lose potency (aminoglycosides, quinolones, macrolides), while other agents may appear to have excessive activity (tetracycline). If the pH is >7.4, the opposite results may occur.

FIG. 1. McFarland standards (left to right) 0.5, 1.0, 2.0, 3.0, positioned in front of a Wickerham card. McFarland standards are used to prepare bacterial suspensions to a specified turbidity. In the Kirby-Bauer disk diffusion susceptibility test protocol, the bacterial suspension of the organism to be tested should be equivalent to the 0.5 McFarland standard.

PROTOCOL

Preparation of Mueller-Hinton plate

1. Allow a MH agar plate (one for each organism to be tested) to come to room temperature. It is preferable to allow the plates to remain in the plastic sleeve while they warm to minimize condensation.

2. If the surface of the agar has visible liquid present, set the plate inverted, ajar on its lid to allow the excess liquid to drain from the agar surface and evaporate.

4. Adjust the turbidity of this suspension to a 0.5 McFarland standard by adding more organism if the suspension is too light or diluting with sterile saline if the suspension is too heavy.

5. Use this suspension within 15 minutes of preparation.

Additional Notes

Inoculum preparation

Organisms to be tested must be in the log phase of growth in order for results to be valid. It is recommended that subcultures of the organisms to be tested be made the previous day.

Never use extremes in inoculum density. Never use undiluted overnight

B

FIG. 3. Kirby-Bauer disk diffusion susceptibility test protocol, inoculation of the Mueller-Hinton agar plate. Step 3. (A) Inoculate the plate with the test organism by streaking the swab in a back-and-forth motion very close together as you move across and down the plate. Rotate the plate 60° and repeat this action. Rotate the plate once more and repeat the streaking action. This ensures an even distribution of inoculum that will result in a confluent lawn of growth. (B) Diagram illustrating the pattern the swab should follow as it is drawn across the plate.

FIG. 4. Kirby-Bauer disk diffusion susceptibility test protocol, inoculation of the Mueller-Hinton agar plate. Step 4. After streaking the Mueller-Hinton agar plate as described in Step 3, rim the plate with the swab by running the swab around the edge of the entire the plate to pick up any excessive inoculum that may have been splashed near the edge.

arrow indicates the path of the swab.

Placement of the antibiotic disks

1. Place the appropriate antimicrobial-impregnated disks on the surface of the agar, using either forceps to dispense each antimicrobial disk one at a time, or a multidisk dispenser to dispense multiple disks at one time. (See steps a. through d. for the use of the multi-disk dispenser or steps e. through g. for individual disk placement with forceps.

a. To use a multidisk dispenser, place the inoculated MH agar plate on a flat surface and remove the lid (Fig. 5).

b. Place the dispenser over the agar plate and firmly press the plunger once to dispense the disks onto the surface of the plate.

c. Lift the dispenser off the plate and using forceps sterilized by either cleaning them with an alcohol pad or flaming them with isopropyl alcohol, touch each disk on the plate to ensure complete contact with the agar surface. This should be done before replacing the petri dish lid as static electricity may cause the disks to relocate themselves on the agar surface or adhere to the lid (Fig. 6d).

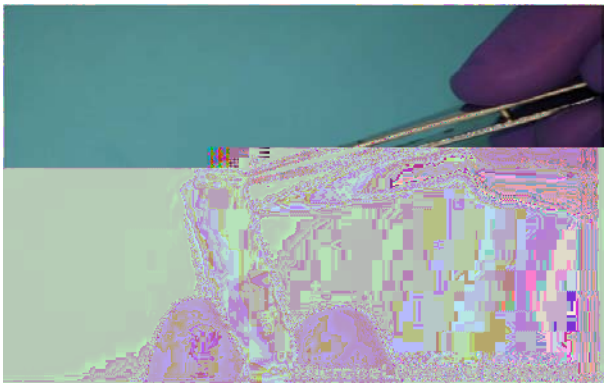
d. Do not move a disk once it has contacted the agar surface even if the disk is not in the proper location, because some of the drug begins to diffuse immediately upon contact with the agar.

e. To add disks one at a time to the agar plate using forceps, place the MH plate on the template (Fig. 7) provided in this procedure (Fig. 6a). Sterilize the forceps by cleaning them with a sterile alcohol pad and allowing them to air dry or immersing the forceps in alcohol then igniting.

f. Using the forceps carefully remove one disk from the cartridge (Fig. 6b).

g. Partially remove the lid of the petri dish. Place the disk on the plate over one of the dark spots on the template and gently press the

A



B

C

D

FIG. 6. Kirby-Bauer disk diffusion susceptibility test protocol, placement

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FIG. 9. Kirby-Bauer disk diffusion susceptibility test protocol, measuring zone sizes. (A) Using su)g

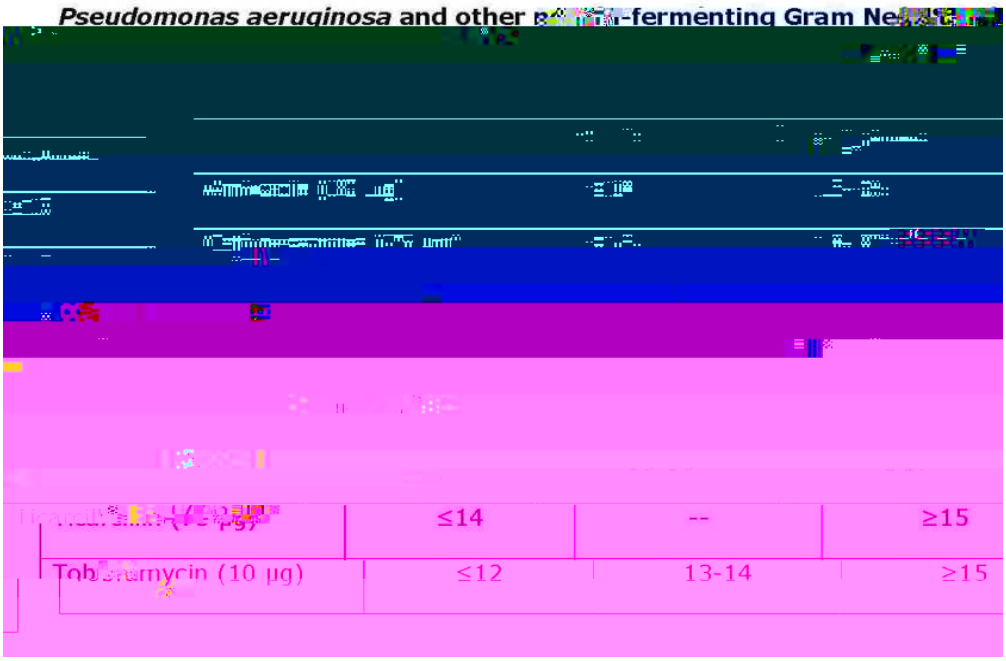
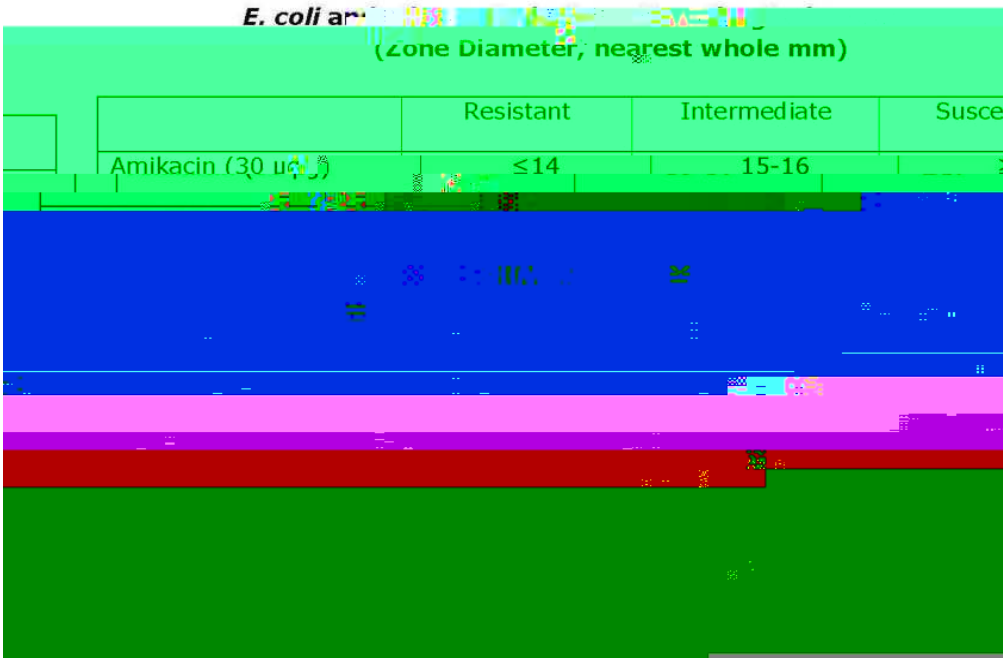


TABLE 3. Zone diameter interpretative standards for E. coli and other enteric gram-negative rods (3)



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