Comparative Study between Kirby Baurer Disk Diffusion with that of Epsilometer Test for Vancomycin Resistance Enterococcus

Dr. Santhoshini Vajjinath¹, Dr. Sangeetha. S², Dr. Sendil Kumar³
Department of Microbiology
Rajarajeswari Medical College & Hospital, Bengaluru, Karnataka, India

Abstract:

**Background:** The Kirby-Bauer disk diffusion test helps determine the susceptibility of a microorganism to various antimicrobial drugs. However, the zones of inhibition measured must be correlated to known standards to determine susceptibility and resistance, and do not provide information on bactericidal versus bacteriostatic activity, or allow for direct comparison of drug potencies. Antibiograms are useful for monitoring local trends in antimicrobial resistance/susceptibility and for directing appropriate selection of empiric antibacterial therapy. There are several laboratory methods available for determining the MIC of an antimicrobial drug against a specific microbe. The MBC can also be determined, typically as a follow-up experiment to MIC determination using the tube, agar, micro dilution method.

**Aims & Objectives:** To compare DDM with MIC epsilometer to determine the VRE from Enterococcal isolates.

**Materials & Methods:** Total 180 Enterococcal isolates were studied. Identification was done by conventional biochemical methods. Antibiotic susceptibility testing was done by Kirby-Bauer disc diffusion and E-strip method on Mueller–Hinton agar and results were interpreted as per CLSI guidelines 2020.

**Results:** among 180 Enterococcal isolates, 12(6.7%) isolates were VRE in DDM while in comparision with E-strip method which yielded 0(0%) VRE.

**Conclusion:** This study shows DDM is a screening method not a confirmatory method to report VRE isolates.

**Keywords:** Vancomycin resistance Enterococcus (VRE), minimum inhibitory concentration (MIC), minimal bactericidal concentration (MBC), disc diffusion method (DDM), E-strip- epsilometer strip.

I. INTRODUCTION

Diffusion methods include. Agar disc diffusion assay, developed in 1940, is one of the oldest methods for routine AST and remains one of the most popular manual techniques for AST in clinical microbiology. The main advantages are simplicity, reproducibility, ease in modifying antimicrobial discs, the possibility for use as a screening test against numerous isolates, and last but not least low cost. Mueller–Hinton agar plates (90 mm diameter) are inoculated with a standardized inoculum of the test micro-organism (corresponding to 0.5 McFarland turbidity standard). Up to 12 commercially prepared paper discs (approximately 6 mm in diameter) with desired concentrations of the tested agent are placed on the inoculated agar surface. Agar plates are incubated under suitable conditions, typically for 16–24 h at 35–37°C. The diameter of the growth inhibition zones around each antibiotic disc is then measured in millimetres and the diameter of the disc is also included in the result. This is performed manually using a sliding caliper or a ruler which is held on the back of the inverted agar plate. The disc diffusion test provides qualitative results by categorizing the bacterial susceptibility as susceptible, or resistant.

**Antimicrobial gradient method**

The Etest is an alternative method used to determine MIC. Similar to the Kirby-Bauer assay, a confluent lawn of a bacterial isolate is inoculated onto the surface of an agar plate. Rather than using circular disks impregnated with one concentration of drug, however, commercially available plastic strips that contain a gradient of an antibacterial are placed on the surface of the inoculated agar plate as the bacterial inoculum grows; antibiotic diffuses from the plastic strips into the agar and interacts with the bacterial cells. Because the rate of drug diffusion is directly related to concentration, an elliptical zone of inhibition is observed with the Etest drug gradient, rather than a circular zone of inhibition observed with the Kirby-Bauer assay. To interpret the results, the intersection of the elliptical zone with the gradient on the drug-containing strip indicates the MIC. Because multiple strips containing different antimicrobials can be placed on the same plate, the MIC of multiple antimicrobials can be determined concurrently and directly compared. E-strip method combines the principles of the dilution and diffusion methods. The Etest MIC Test Strip is a commercially available. The agar medium is previously inoculated with a bacterial lawn of the tested micro-organism. Thin test strips which are impregnated with an increasing concentration gradient of the dried antimicrobial drug from one end to the other, are then laid on the agar surface. After overnight incubation, the MIC values are read by viewing the strips from the top of the petri dish and determined at the point of intersection of the strip with an elliptical growth inhibition zone.This test is rapid, easy to use routinely but is relatively expensive method. Hence, this approach becomes costly if more than a few drugs are tested. The method is best applicable for only one or two drugs and for fastidious, multidrug resistance organism. This method can be used for the determination of antibacterials, antifungals and antimycobacterials. Other methods like Dilution methods -
II. MATERIALS

It is a retrospective study conducted after taking approval from ethical committee. All Enterococcal isolates from clinical samples were included in this study, for 5 months from January to May 2020

III. METHODOLOGY

All 180 Enterococcal isolates from clinical samples were processed as per standard protocol CLSI guidelines 2020. A suspension of VRE ATCC 51299 and the test VRE organism in sterile nutrient broth of 0.5 McFarland standard using isolated colonies was prepared. A lawn culture was made on the surface of the MHA agar plate using sterile cotton swabs. Vancomycin (VA) 30μg antibiotic discs were placed. The plates were incubated at 37 degree for 18-24 hours. After 24 hours, the zone of inhibition was recorded. A clear zone of inhibition around the disc indicates sensitivity and their absence indicates resistance. The diameter of each zone including the diameter of the disc was measured and recorded in millimeters and the result was then compared with the zone size interpretative chart. Etest-Minimum inhibitory concentration (MIC) determination was done by Vancomycin E test strips (Hi-media Laboratory, Mumbai). Test was performed on Mueller-Hinton agar plates as per the manufacturer’s instructions. MIC values ≤4 μg/ml was taken as susceptible and ≥32 μg/ml as resistant.

Results: In 180 Enterococcal isolates, 12(6.7%) isolates were VRE in DDM while on comparison with E-strip method which yielded 0(0%) VRE.

IV. CONCLUSION

There are multiple factors that determine the size of a zone of inhibition in this assay, including drug solubility, rate of drug diffusion through agar, the thickness of the agar medium, and the drug concentration impregnated into the disk. Due to a lack of standardization of these factors, interpretation of the Kirby-Bauer disk diffusion assay provides only limited information on susceptibility and resistance to the drugs tested. The assay cannot distinguish between bacteriostatic and bactericidal activities, and differences in zone sizes cannot be used to compare drug potencies or efficacies. Comparison of zone sizes to a standardized chart will only provide information on the antibacterial to which a bacterial pathogen is susceptible or resistant. The MIC was determined at the point where inhibition of the growth intersected with Etest strip. The present study shows that disc diffusion remains an unreliable susceptibility testing method for vancomycin drug resistance. This study shows E-test should be used as confirmatory method to report VRE, as it also recommend under CLSI 2020 guidelines.

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