IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 7, Issue 3 (Jul. – Aug. 2013), PP 29-31
www.iosrjournals.org

E-test for Detection of Antimicrobial susceptibility of Vancomycin (Glycopeptides) in Methicillin Resistant Staphylococcus aureus (MRSA)

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Abstract : *E-test is a quantitative technique for determining the antimicrobial susceptibility of gram positive and gram negative bacteria. The system comprises a predefined antibiotic gradient which is used to determine the MIC (Minimum Inhibitory Concentration), in µg /ml of different antimicrobial agents against microorganisms as tested on agar media using overnight incubation. MIC of a given antibiotic in µg /ml that will inhibit the growth of a particular bacterium under defined experimental conditions. E-test directly quantifies antimicrobial susceptibility in terms of discrete MIC values. However in using a predefined stable and continuous antibiotic concentration gradient, E-test MIC values can be more precise and reproducible than results obtained from conventional procedures. E-test microbial concentration gradient is preformed, predefined and stable and is not dependent on diffusion. MIC of 0.5 µg /ml, 1 µg /ml and 1.5 µg/ml were observed when Vancomycin E-test was used to determine the glycopeptide susceptibility in MRSA (Methicillin Resistant Staphylococcus aureus).*

Keywords: *Epsilometer, MIC, BSAC, AST, Susceptibility, MRSA, Vancomycin.*

I. Introduction

The principle of the epsilometer test was first described in 1988 and was introduced commercially in 1991 by AB Biodisk. The Etest method (AB Biodisk, Solna, Sweden) gives an MIC result and is affected by test conditions in a similar way to other MIC and diffusion methods. Evaluations comparing the Etest with dilution MIC and molecular methods have generally found good essential agreement. The Etest has an advantage over other MIC methods. In this it is easy to set up as a disc diffusion test. It is a quantitative test technique, for determining the minimum inhibitory concentration MIC of Anti microbial agents, against micro organisms and for detection of resistance mechanisms.

The Etest gradient technology is based on a combination of the concepts of dilution and diffusion principles for susceptibility testing. As with other dilution methods, Etest directly quantifies antimicrobial susceptibility in terms of discrete MIC values. The Etest is basically an agar diffusion method. It consists of plastic strip calibrated with a MIC scale in µg/ml and codes to identify the antimicrobial agent. A predefined concentration gradient antibiotic across 15 two–fold dilutions of a conventional MIC method, is immobilized on the other surface of the carrier. When Etest strip is applied on to an inoculated agar surface the preformed exponential gradient of antimicrobial agent or reagents are immediately transferred to the agar matrix. MIC is read directly from the scale in terms of µg/ml at the point where the edge of the inhibition ellipse intersects the strip other growth patterns may also be seen for resistance detection methods.

Although processed like the disc diffusion test i.e. similar inoculums preparation, choice of agar media and incubation conditions. The Etest is a preformed, predefined, stable and it is not dependent on diffusion. To obtain reproducible MICs from a gradient based system, a stability of the gradient must be maintained throughout the critical period when the position of the growth/inhibition edge for a particular bacterium/antibiotic combination is determined due to the stability and precision of the Etest predefined gradient MIC values have been shown to be reproducible and equivalent to those of dilution procedures. The Etest utilizes a rectangular strip that has been impregnated with the drug to be studied. A lawn of bacteria is spread and grown on an agar plate, and the Etest strip is laid on top; the drug diffuses out into the agar, producing an exponential gradient of the drug to be tested. There is an exponential scale printed on the strip. After 24 hours of incubation, an elliptical zone of inhibition is produced and the point at which the ellipse meets the strip gives a reading for the (MIC) of the drug.

II. Evaluation Of E-Test

A. Materials and Methods:

Samples are collected from tertiary care hospital in south India for a period of six months i.e. 2011 March to August 2011. Isolates of MRSA are checked for the susceptibility to Vancomycin. MIC is

determined by performing E test following BSAC guidelines on Muller Hinton agar plate. Ensure that whole strip is in complete contact agar surface don't place the strip upside down as no inhibition ellipse will form since the antibiotic will not diffuse across the non porous plastic strip.

B. Storage:

Etest should always be stored according to the temperature specified (-20^{o}C), left over Etest strips from an opened package must be kept dry by storing them with active desiccant in an air tight container. Prevent moisture from penetrating into or forming within the storage container. Etest strips must be kept dry.

C. Inoculum preparation:

Emulsify several well isolated colonies of MRSA from a pure overnight culture into a suitable suspension medium. Fastidious organisms should be suspended in broth and used within 15 minutes. Compare the turbidity to the appropriate 0.5 Mc farland standard.

D. Inoculation:

Dip a sterile cotton swab to the inoculum suspension and press against the inside wall of the tube to remove excess fluid carefully streak the entire agar surface evenly in three directions. Allow excess moisture to be fully absorbed and ensure that the surface is completely dry before applying Etest strips. When the inoculum and inoculation are optimal and even confluent growth will be obtained.

E. Application of strips:

Apply the Etest strip to the agar surface with the MIC scale facing upwards. This can be done using forceps. Ensure that the whole strips is in complete contact with the agar surface. Don't place the strip upside down as no inhibition ellipse will form since the antibiotic will not diffuse across the non-porous plastic strip. If air pockets are seen under the strip remove them by pressing gently on the strip with the applicator tip or forceps working from the lowest concentration upwards. Small bubbles will not affect results. Once applied the strip cannot be moved because of instantaneous release of antibiotic into the agar.

F. Incubation & Reading:

Plates are incubated in an inverted position at 37 ^{0}c for overnight incubation. Read the MIC value where the edge of inhibition ellipse mean intersects the strips

G. Interpretation:

MIC breakpoint for defining susceptibility categories as provided could be used for interpreting Etest MIC values. Etest strips of Vancomycin which is one of the glycopeptide is used to check the susceptibility.

III. Result

All isolates were found to be sensitive to Vancomycin with MICs of 0.5 µg, 0.75µg, 1.0 µg and 1.5 µg according BSAC (British Society for Antimicrobial Chemotherapy).

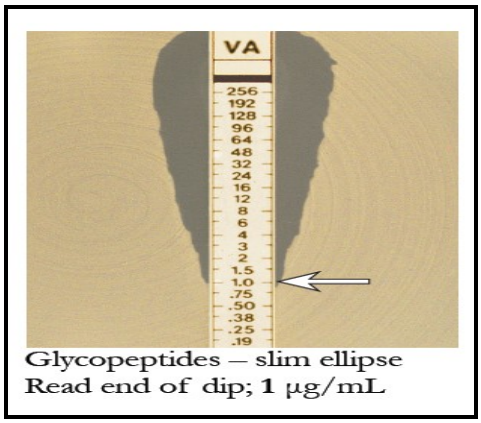

Fig.1 Glycopeptides

IV. Conclusion

All the isolates which are processed for observing Glycopeptide Resistance (Vancomycin) were found to be susceptible to Vancomycin. No VRSA (Vancomycin Resistant Staphylococcus aureus) strains were observed.

References

[1]. FDA Draft, Review Criteria for Assessment of Antimicrobial Susceptibility Testing Devices food and drug administration, Division of Clinical laboratory Devices May 1991, Revised February 2003.
[2]. A novel Technique for direct quantification of Antimicrobial susceptibility of Micro organisms Bolmstron, A. et al.
[3]. Evaluation of E test a novel Method quantifying antimicrobial activity journal of Antimicrobial chemotherapy
[4]. Schulz J.E. et al. (1993). Reliability of the E test for detection of Vancomycin and high-level Amino glycosides resistances in Enterococcus species. Journal of Clinical Microbiology.
[5]. Citron D.M. et al. (1991). Evaluation of E test for susceptibility testing of Anaerobic bacteria. Journal of Clinical Microbiology.
[6]. CLSI 2006. Methods for dilution Anti microbial susceptibility test for bacterial that grow aerobically.

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