

Minimum Inhibitory Concentration of Staphylococcus Aureus against Vancomycin

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Abstract - The main objective of this investigation is to find out the minimum inhibitory concentration (MIC) of Staphylococcus aureus with reduced inclination to vancomycin among 30 clinical isolates of methicillin-resistance Staphylococcus aureus (MRSA) and was screened by Etest and broth dilution methods. The comparative analysis of MIC for vancomycin by broth dilution and Etest methods discloses that both methods were suitable and was confirmed by the two-tailed P value which was statistically significant.

Keywords – Staphylococcus aureus, vancomycin, Etest and broth dilution method

I. INTRODUCTION

Staphylococcus aureus is a key causative of many human infections in skin and bone [1]. In 1880, Alexander Ogston for the first time isolated *Staphylococcus aureus* from a surgical blister and narrated the task of *S. aureus* in local infections. In 1958, an antibiotic named vancomycin [2], made of glycopeptides was introduced from *Streptomyces orientalis* and was widely used in the alimient of infectivity by Gram-positive bacteria especially *S. aureus*. In the last 20 years, vancomycin usage was increased dramatically for treating the illness caused by Gram-positive bacteria since they were insensitive to other antibiotics of less toxicity. Vancomycin enters the cell membrane of Gram-positive bacteria and destroys its production which in turn inhibits its activity whereas in case of Gram-negative bacteria it was not possible to enter the outer membrane [3,4]. Alike, vancomycin was an active antibiotic against the infections caused by the species of *Listeria*, *Streptococcus*, *Actinomyces* and *Lactobacillus* in turn the combination of vancomycin and an aminoglycoside acts against *Staphylococcus* sp., *Streptococcus* sp. and *Enterococci* sp.[5,6,7]

The minimum inhibitory concentration (MIC) could be used as a comparative measure of antimicrobial activity against a particular pathogen and is the lowest concentration of an antimicrobial agent that inhibits the visible growth of micro organisms after overnight invitro incubation [8,9]. Based on MIC guidance, bacteria were categorized into vulnerable, of halfway resistance or resistance to a particular antimicrobial agent.

The main objective of this investigation is to find out the MIC of *Staphylococcus aureus* with reduced inclination to vancomycin among 30 clinical isolates of methicillin-resistance *Staphylococcus aureus* (MRSA) and was screened by Etest and broth dilution methods.

II. MATERIALS AND METHODS

A. Collection of samples –

Control strains (*Staphylococcus aureus*). 30 strains of methicillin resistant *Staphylococcus aureus* species were collected from the patients in the Global hospital and health city.

B. Isolation and identification –

For isolation and identification of morphology of *S. aureus*, conventional techniques like Gram staining and biochemical tests were performed.

C. Etest –

Etest macro method values were determined by using vancomycin Etest strips [10].

D. MIC determination –

According to clinical laboratory standards institute, MICs for vancomycin was verified by both Etest and broth dilution methods. Quality of the strains was carried out using clinical laboratory standards institute suggested reference strains.

E. Data analysis –

The statistical significance among *S. aureus* and MICs were identified using the two-sided chi-square test.

III. RESULTS AND DISCUSSION

A. Etest vancomycin MIC test –

The strains obtained from the patients were standardized to 0.5 McFarland turbidity and plated on Mueller-Hinton agar, supplemented with 2% NaCl. Etest strips were placed and allowed to be incubated for 24 h at 35°C.

CDC has adopted three criteria to identify vancomycin-intermediate *S. aureus* strains [11,12,13]:

1. Broth microdilution vancomycin MIC of 8-16µg/mL
2. Etest vancomycin MIC of >6µg/mL and
3. Growth on MH agar containing 6µg/mL vancomycin within 24 hours.

Based on the said reports the present study showed that 27 strains out of 30 strains grown on vancomycin plates were found to be sensitive; 1 as intermediate; 1 as resistances and the last was found to be MIC Creep in criteria (Fig. 1). The present analysis insists the need of continuous prudence to be maintained in order to preserve the precious antibiotic vancomycin to treat vancomycin-intermediate *S. aureus* and to prolong its therapeutic usefulness.

Fig. 2 shows the comparative analysis of MIC for vancomycin by broth dilution and Etest methods. It discloses that both Etest and broth dilution methods were more appropriate in predicting the active nature of vancomycin against vancomycin-intermediate *S. aureus*. The present study revealed that there was no major variation in the incidence of vancomycin MICs in *S. aureus* isolates from dissimilar unwearied populations. Similar results were observed by Wang et al. [14] in their work on increased vancomycin MICs for *Staphylococcus aureus* clinical isolates from a university hospital.

B. Two Way Table

i. McNemar's test -

Table 1 shows the two way table of MIC for both broth dilution and Etest method using McNemar's test. McNemar's test was generally used to evaluate the magnitude of equal values. It also helps in the analysis of control and sample values and each sample was harmonized to a particular control. Else, it could be used to examine the experimental studies of two treatments of same subject [12].

ii. P Value -

The two-tailed P value equals 0.4795 by conventional criteria and it was considered as statistically significant. Based on the continuous correction of McNemar's test, the P value was calculated. Chi squared equals 0.500 with 1°of freedom. The P value revealed the good correlation among the broth dilution and Etest methods with MIC results [14, 15].



Fig. 1 Etest Strips on MH Agar

Table 1. The two way table of MIC for broth dilution and Etest method

E Test \ Broth dilution	Broth dilution		Total
	+	-	
+	29	0	29
-	3	0	3
Total	32	0	32

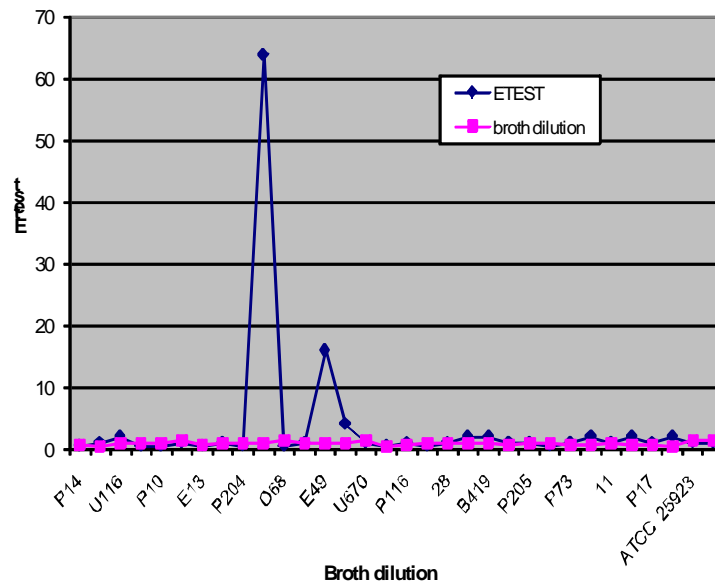


Fig. 2 Comparison of MIC for vancomycin by broth dilution and Etest methods.

IV. CONCLUSION

Minimum inhibitory concentration of *Staphylococcus aureus* for vancomycin from methicillin resistant species isolated from the patients was analysed using Etest and broth dilution methods. The present study showed that 27 strains out of 30 strains grown on vancomycin plates were found to be sensitive; 1 as intermediate; 1 as resistances and the last was found to be MIC in criteria that shift population of *Staphylococcus aureus* overtime. The comparative analysis of MIC for vancomycin by broth dilution and Etest methods discloses that both Etest method and to broth dilution method were more appropriate. The two-tailed P value was considered as statistically significant. Hence both methods were recommended for the treatment of methicillin resistant *Staphylococcus aureus* isolates.

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