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Research Article

COMPARISON OF ETEST AND AGAR DILUTION FOR DETERMINING MINIMUM INHIBITORY CONCENTRATION OF VANCOMYCIN TO HEALTHCARE-ASSOCIATED METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

Objectives: To compare agar dilution method and Etest in the determination of minimum inhibitory concentration (MIC) of vancomycin to healthcare-associated methicillin-resistant *Staphylococcus aureus* (HA-MRSA).

Methods: A total of 98 non-duplicate strains of HA-MRSA isolated from different clinical specimens were tested for their antibiotic susceptibility pattern by Kirby-Bauer disk diffusion method and vancomycin MIC by agar dilution method and Etest (BioMerieux, France).

Results: Out of 98 strains of HA-MRSA, 94 (95.9%) were vancomycin susceptible (MIC ≤2 µg/ml and 4 (4.1%) were vancomycin intermediate (MIC 4 µg/ml) by agar dilution method. By Etest, 53 (54.1%) were vancomycin susceptible, 4 (4.1%) were vancomycin intermediate, and the remaining 41 isolates had vancomycin MIC between 2 µg/ml and 4 µg/ml.

Conclusion: Etest allows the detection of HA-MRSA strains with intermediate MIC values in addition to traditional dilutions. These properties will help in detection of MIC creep and also decision-making in using vancomycin for the treatment of serious infections caused by HA-MRSA.

Keyword: Vancomycin, Minimum inhibitory concentration, Etest, Agar dilution.

INTRODUCTION

Healthcare-associated methicillin-resistant *Staphylococcus aureus* (HA-MRSA) has emerged as a significant pathogen all over the world, causing a variety of infections in hospitalized patients. HA-MRSA usually exhibits multidrug resistance, leaving vancomycin as an option for treatment of serious infections such as bacteremia, osteomyelitis, and pneumonia [1]. Vancomycin use has increased since the mid-1980s, exerting antibiotic pressure on *S. aureus.* This could have been responsible for the emergence of vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus*. VISA may include homogenous VISA or heterogeneous VISA [2]. In addition to these, recently there has been a concern in the increase in vancomycin minimum inhibitory concentration (MIC) even within the susceptible range. This phenomenon is known as "MIC creep [2-4]. Therefore, accurate determination of vancomycin MIC is crucial before selecting it for treatment. There are several methods used for this purpose, which vary in their sensitivity and specificity [5-8]. The objective of this study was to compare agar dilution and Etest in the determination of vancomycin MIC to HA-MRSA.

METHODS

Study design and setting

This cross-sectional study was carried out over a period of 2 years (January 2014-December 2015) using clinical specimens collected from four tertiary care hospitals in coastal Karnataka, India. The study had the approval of Institutional Ethics Committee.

Bacterial strains

A total of 98 non-repetitive strains of HA-MRSA were studied. Infections were considered healthcare-associated if they evolved at least 48 hrs after hospital admission [9]. Standard bacteriological methods were used for the isolation and identification of *S. aureus* [10]. Cefoxitin disk (30 µg) diffusion was used in the identification of MRSA [11]. HA-MRSA strains were preserved in trypticase soy broth with 20% glycerol at –80°C [10].

Antibiotic susceptibility testing

The antibiotic susceptibility testing was performed using Kirby-Bauer disk diffusion method, and results were interpreted as per Clinical Laboratory Standard Institute (CLSI) guidelines [11]. The antibiotics tested included ciprofloxacin (5 µg), clindamycin (2 µg), erythromycin (15 µg), gentamicin (10 µg), linezolid (30 µg), rifampicin (5 µg), teicoplanin (30 µg), tetracycline (30 µg), and trimethoprim/sulfamethoxazole (1.25 µg/23.75 µg). *S. aureus* ATCC 25923 was used as the control. The antibiotics were purchased from HiMedia Laboratories, Mumbai, India.

Determination of MIC of vancomycin by agar dilution method

MIC of vancomycin was determined by agar dilution method using CLSI guidelines [12]. Mueller-Hinton agar plates with a gradient concentration of vancomycin (0.125-128 µg/ml) were prepared. Two to three colonies grown on blood agar were picked and inoculated into Mueller-Hinton broth (MHB) and incubated at 35°C for 4-6 hrs. The turbidity was matched with McFarland 0.5 standard (bacterial count approximately 1.5×10^8 cfu/ml) and diluted 1 in 10 in sterile MHB (bacterial concentration 1.5×10^7 cfu/ml). 2 µl of the suspension was then spot inoculated on MHA plates with different concentration of vancomycin and incubated at 35°C for 24 hrs. The lowest concentration of vancomycin that inhibited bacterial growth was considered MIC.

Determination of MIC of vancomycin by Etest

Etest was performed according to the manufacturer's instructions (BioMerieux, France). The vancomycin concentration range was 0.016 µg/ml 256 µg/ml. MRSA colonies (3-4) grown overnight on blood agar were used to prepare a bacterial suspension of a McFarland 0.5 standard in sterile water. This suspension was spread on MHA. The vancomycin Etest strip was placed on the inoculated agar plate, and the plate was incubated at 35°C for 24 hrs and readings were taken.

Vancomycin susceptible controls used were *S. aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212. *E. faecalis* ATCC 51299 was used as a vancomycin-resistant control in both agar dilution and Etest.

Interpretation of MIC of vancomycin determined

CLSI guidelines were used for interpretation of vancomycin MIC determined by both Etest and agar dilution method [11]. HA-MRSA strains with vancomycin MIC ≤2 µg/ml were considered vancomycin susceptible, 4-8 µg/ml were considered vancomycin intermediate, and ≥16 µg/ml as vancomycin resistant.

RESULTS

A total of 98 HA-MRSA strains isolated from clinical specimens collected from patients with different clinical conditions were used in this study (Table 1).

Maximum resistance was observed to ciprofloxacin followed by erythromycin, trimethoprim/sulfamethoxazole, clindamycin, gentamicin, and tetracycline (Table 2).

Table 1: Clinical conditions caused by HA-MRSA

Type of infection	HA-MRSA (%) (n=98)
Surgical site infection	21 (21.43)
Cellulitis	19 (19.39)
Catheter associated bacteremia	14 (14.29)
Infected bed sore	11 (11.22)
Infected ulcer	11 (12.24)
Burn wound infection	08 (08.16)
Infected toxic epidermal necrolysis	03 (03.06)
Bacteremia	07 (07.14)
Psoas abscess	01 (01.02)
Empyema	01 (01.02)
Pneumonia	01 (01.02)
Osteomyelitis	01 (01.02)
Total	98 (100.00)

HA-MRSA: Healthcare-associated methicillin resistant *Staphylococcus aureus*

Table 2: Antibiotic resistance pattern of HA-MRSA

Antibiotic	HA-MRSA (%) (n=98)
Ciprofloxacin	77 (78.57)
Clindamycin	47 (47.96)
Erythromycin	64 (65.31)
Gentamicin	39 (39.80)
Linezolid	00 (00.0)
Rifampicin	01 (01.02)
Teicoplanin	00 (00.0)
Tetracycline	34 (34.69)
Trimethoprim/sulphamethoxazole	53 (54.08)

HA-MRSA: Healthcare-associated methicillin resistant *Staphylococcus aureus*

Table 3: Vancomycin MIC of HA-MRSA by agar dilution method and Etest

Vancomycin MIC µg/ml	Number of HA-MRSA (%) (n=98)	
	Agar dilution	E test
0.5	11 (11.2)	0
0.75	-	1 (1)
1	37 (37.8)	11 (11.2)
1.25	-	1 (1)
1.5	-	16 (16.3)
1.75	-	2 (2)
2	46 (46.9)	22 (22.4)
2.5	-	25 (25.5)
3	-	16 (16.3)
3.5	-	0
4	4 (4.1)	4 (4.1)

MIC_{90} and MIC_{50} by agar dilution method=2 µg/ml; MIC_{90} and MIC_{50} by Etest=3 µg/ml and 2 µg/ml respectively. HA-MRSA: Healthcare-associated methicillin resistant *Staphylococcus aureus*, MIC: Minimum inhibitory concentration

Out of 98 HA-MRSA strains, 94/98 (95.9%) and 53 (54.1%) were vancomycin susceptible (MIC ≤2 µg/ml) by agar dilution method and Etest, respectively. Four strains were vancomycin intermediate (MIC 4 µg/ml) by both the methods. 41 strains of HA-MRSA showed vancomycin MIC >2 µg/ml but <4 µg/ml (Table 3). MIC determination by Etest was 0.5-1 µg/ml more compared with the values obtained by agar dilution method. Out of 4 strains of VISA, 2 were isolated from bacteremia cases and the remaining from the wound.

DISCUSSION

Different methods available to determine the vancomycin MIC to *S. aureus* vary in their sensitivity and specificity [13,14]. Broth microdilution (BMD) recommended by the CLSI is considered to be the gold standard. Agar dilution method is also recommended. The automated methods and disk diffusion test may not produce reliable results [2,5-7,13-16].

In this study, the majority of HA-MRSA strains were vancomycin susceptible by agar dilution (95.9%) and Etest (54.1%). Although vancomycin resistance was not detected, 4 (4.1%) strains were vancomycin intermediate (VISA, vancomycin MIC 4 µg/ml). This is a matter of concern. Further, vancomycin MIC determined by Etest was consistently 0.5-1 µg/ml more than that determined by agar dilution method. Similar observations were made by previous researchers also [6-8,14-16]. This difference could be due to the differences in the concentration of vancomycin used in these tests. Agar dilution/BMD uses vancomycin in geometric progression/double dilutions, whereas Etest uses the drug in arithmetic progression including the intermediate concentrations also. Therefore, if one wants to investigate for small changes/differences in vancomycin MIC, including MIC creep Etest should be preferred.

Pharmacodynamic studies have shown that the area under the vancomycin concentration to MIC ratio (AUC/MIC) is required to predict the effectiveness of vancomycin treatment for serious infections caused by MRSA [17,18]. AUC/MIC of 400 or more has been advocated to achieve the clinical effectiveness of vancomycin therapy [19,20]. The probability of achieving this ratio is almost 100% only if the MRSA has vancomycin MIC ≤0.5 µg/ml; and the probability is almost 0% if the isolate has MIC 2 µg/ml [4,21]. Therefore, even minor differences in vancomycin MIC could affect AUC/MIC and the outcome of vancomycin treatment. Etest is suited for determining such minor intermediate changes in vancomycin MIC.

To conclude, although dilution methods are gold standard for determination of vancomycin MIC, they cannot determine the MIC values in the intermediate zone. Etest can be used to determine vancomycin MIC in the intermediate zone, minor changes in MIC and study "MIC creep". It may also be useful in predicting the outcome of vancomycin treatment.

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