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Short report

Comparison of broth microdilution and E-test for susceptibility of MRSA to vancomycin

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Abstract

Introduction: Healthcare-associated methicillin-resistant *Staphylococcus aureus* (MRSA) developed soon after the introduction of methicillin. These bacteria have shown resistance to multiple drugs and therefore vancomycin became the antibiotic of choice for treatment of MRSA infections. Vancomycin is a bactericidal antibiotic that acts by inhibiting bacterial cell wall synthesis. This study aimed to compare broth microdilution (BMD) and E-test in determining vancomycin minimum inhibitory concentration (MIC) against MRSA.

Methods: A total of 30 clinical isolates of MRSA were acquired from Colombo South Teaching Hospital, Sri Lanka. These MRSA strains were identified by cefoxitin disk diffusion and the vancomycin MIC was determined through BMD and E-test. The Kruskal-Wallis test was applied to test if the samples originated from the same distribution with post hoc determination of the correlation between methods using the Mann-Whitney U test.

Results: All 30 MRSA isolates were 100% vancomycin susceptible (≤2 μg/mL) irrespective of methodology, according to the Clinical and Laboratory Standards Institute (CLSI) established breakpoints. However, the E-test MIC values were 1 to 2 dilutions higher than those of BMD. A statistically significant difference between vancomycin MICs of BMD and E-test ($p = <0.00001$) was calculated which indicated a difference in accuracy.

Conclusion: Due to cost, convenience, and the ability to detect vancomycin intermediate *Staphylococcus aureus* (VISA), exploring the possibility of using E-test as an alternative to BMD is worthwhile.

Keywords: *Vancomycin, BMD, E-test, MRSA, MIC*

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tiIntroduction

Staphylococcus aureus is a Gram-positive bacterium that causes life threatening infections including bacteraemia, osteomyelitis, infective endocarditis, and pneumonia.[1] Although Penicillin came into use against *S aureus* infections, resistance developed against penicillin by the late 1950s. This led to the development of methicillin, a semi-synthetic form of penicillin in 1959. Unfortunately, methicillin-resistant *S. aureus* (MRSA) came into being soon after the introduction of the antibiotic.[2] Vancomycin, a bactericidal antibiotic that inhibits bacterial cell wall synthesis became the drug of choice against MRSA since its discovery in 1961. Due to the extensive use of vancomycin, MRSA strains developed mechanisms to reduce their susceptibility. This leads to the question as to whether vancomycin can still be used as a treatment option against MRSA.[1,3]

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that is needed to completely inhibit the growth of bacteria and is used to determine susceptibility of bacteria against appropriate antibiotics.[4] According to Foster *et al.* (2017), the 'MIC breakpoint is a chosen concentration in mg/L of an antibiotic that defines whether a species of bacteria is susceptible or resistant to the antibiotic'.[5] If the MIC is less than or equal to the breakpoint it is susceptible and if the MIC is greater than the breakpoint it is considered intermediate or resistant to the antibiotic.[5] Clinical and Laboratory Standards Institute (CLSI, 2018) established susceptibility breakpoints for vancomycin are as follows: susceptible, MIC ≤2 μg/mL; intermediate (VISA), 4–8 μg/mL; and resistant (VRSA), ≥16 μg/mL.[1]

Resistance to vancomycin requires as many as six mutations in different genes which cause structural changes in the cell envelope, reducing access of the drug to its target site. Increased use of vancomycin since the mid-1980s exerted an antibiotic pressure on *S. aureus*, causing development of intermediate/resistance.[5] Vancomycin-intermediate *S. aureus* (VISA) have an MIC of 4-8 μg/mL. Heterogeneous-VISA (hVISA) are strains where most of the population have an MIC of ≤2 μg/mL, but include a subpopulation of cells with MIC of 4-8 μg/mL.[5]
Recently, concerns have arisen due to the increase of vancomycin MIC even in susceptible strains which threaten clinical use of the antibiotic. This event, known as 'MIC creep' has been linked to therapeutic failure as it renders vancomycin ineffective against isolates with an MIC between 1 and 2 μg/mL.[6]

Broth microdilution (BMD) and Epsilometer test (E-test) are accurate methods to calculate MIC and provide quantitative results. BMD is the gold standard test used. However, time and personnel constraints have led to many laboratories to stop using BMD routinely and use automated systems and E strips with variable specificities and sensitivities.[6,7] An E-test strip contains a predefined, exponential gradient of antibiotic concentrations packed within a strip. After incubation for 24-48 hours, an oval-shaped inhibition zone intersects the test strip at the inhibitory concentration of the antibiotic.[8]

Some laboratory methods used to determine MIC values have low accuracy which in turn may lead to poor patient outcomes.[9] Accurate interpretation of MIC results is important as overestimation may lead to the unnecessary use of alternative antibiotics, increasing the possibility of resistance development.[10] Evaluating different MIC test methods against the CLSI recommended gold standard (BMD) would help determine the accuracy of these methods. The

main objective of the current study is to compare the E-test with BMD for the determination of vancomycin MIC of MRSA clinical isolates.

Methods

In this study, we analysed vancomycin MICs of 30 clinical isolates of MRSA obtained from urine, blood, wound and skin swabs, peritoneal and pleural fluids, tracheal aspirates and sputum samples from the Department of Microbiology, Colombo South Teaching Hospital collected from 12th March to 12th May 2018. Isolates were identified according to conventional laboratory techniques (catalase test, Gram stain, slide coagulase) followed by confirmation with the tube coagulase DNase tests. The *S. aureus* isolates were screened for resistance to cefoxitin (30 µg) to identify MRSA (CLSI 2018).[11] The MIC of vancomycin was determined using the broth dilution method (CLSI, 2018) at a range of 512mg/L to 0.25mg/L. *S aureus* ATCC 29213 was used as the control. Vancomycin E-test strips were used by commercially available Vancomycin Ezy MIC™ Strip EM060 (HiMedia, India).

Statistical analysis

Results of MIC determined by BMD and E-test were analysed. Two categorical variables were selected - the MIC between >1 and ≤2 mg/mL and MIC ≤1. Wilcoxon matched pairs signed rank sum test was used. Data processing and analysis were done using Social Sciences Statistics online calculator (*https://www.socscistatistics.com/*). The test was done at 5% significance level.

Results

Table 1: MIC distribution of MRSA isolates (n=30)

MIC (µg/mL)	Number of isolates (%)			
	BMD		E-test	
≤0.25	2	(6.7)	-	
≤0.38	-	-	1	(3.3)
≤0.5	17	(56.7)	-	
≤0.75	-	-	2	(6.7)
≤1	11	(36.7)	16	(53.3)
≤1.5	-	-	10	(33.3)
≤2	-	-	1	(3.3)
ATCC 25923	0.5		1	

Of 107 clinical isolates, 72 were *S. aureus* of which 30 (41.7%) were MRSA. The vancomycin MICs of MRSA for E-test and BMD ranged from 0.38-2 µg/mL and 0.25-1 µg/mL respectively. According to CLSI susceptibility break points, all samples were vancomycin susceptible irrespective of methodology, with MICs ≤ 2 µg/mL as shown in Table 1.

Vancomycin MIC of MRSA isolates

The vancomycin MICs of the isolates varied from 0.25-2 mg/L (Table 1). All isolates had MICs ≤2 mg/L, which is within the sensitive range. All 30 MRSA isolates had an MIC ≤ 1mg/L using the BDS method. Only 20 of the 30 isolates (66%) had an MIC ≤ 1mg/L by the E-test method (Table 2). This difference is statistically significant
($p < 0.00001$).

Table 2: Comparison of MIC (µg/mL) of BMD and E-test

Specimen number	MIC (µg/mL)	
	BMD	E-test
1	0.25	0.38
2	0.25	0.75
3	0.5	0.75
4-19	0.5	1
20-29	1	1.5
30	1	2

Discussion

In this study, all MRSA isolates had MICs ≤2 mg/L by both methods, which is well within the sensitive range Comparing BMD to the E-test, MIC of all MRSA isolates were <1 mg/L while only 20 of the 30 isolates had an MIC of <1mg/L by the E-test. Ten isolates showed an MIC range of between 1mg/L and 2mg/L by the E-test. Similar findings have been reported previously[9,10,12] including those of Prakash, Lewis and Jorgensen (2008) and Sader, Rhomberg and Jones (2009).[13,14] One such study found that 89-98% of MICs were as high as 1.5 or 2 μg/mL using the E-test while only 3 to 12% of the same isolates had an MIC of 2 μg/ml using the BMD.[13]

However, contrasting results were reported by Khatib et al. (2013) where 44.2% of the E-test MICs were equal to BMD and there were isolates which gave lower MICs by the E-test than by BMD (31.4% with ≥ 1 dilution lower MICs).[15] Our study has demonstrated that ten of the 30 isolates had MICs of 1.5 or 2 μg/ml while none had MICs >1mg/L by BMD.

It has been reported that BMD is not very reliable in classifying VISA as 'non-susceptible to vancomycin' and therefore, the identification of VISA needs to be verified by either MicroScan or E-test.[16] Philips et al (2016) proposes a theory that this could be because most BMD MICs are clustered at 0.5 μg/mL as also seen in the present study and its ability to detect strain differences might be a limitation. One possible reason for this could be that the subpopulations of VISA occur at very low frequencies of ≤1 per 10^5 to 10^6 CFU, resulting in a low probability of being detected since the inoculum used in BMD was approximately $5x10^5$ CFU in this study.[10]

Musta *et al* have preferred the E-test over BMD to detect heteroresistant vancomycin intermediate *S. aureus* (h VISA) isolates as E-test is much less labor intensive, less costly and also maintains high specificity and sensitivity.[16] Tenover (2010) suggests that since the E-test results can be read at one-half dilution intervals (1,2,3,4,6 and 8 μg/mL) in contrast to BMD which gives results at two-fold dilution intervals (1,2,4,8,16 μg/mL), the standard E-test is a more accurate predictor of successful treatment of MRSA infections.[17]

A cohort study by Wi et al (2012) on 137 patients with MRSA bacteremia showed that a vancomycin MIC ≥ 1 μg/mL had a significant effect on their mortality. This was also supported by Van Hal et al (2011) in a meta-analysis of 22 papers.[12,18] Van Hal classified the susceptibility of MRSA for vancomycin using a single breakpoint of 1.5 μg/mL regardless of methodology. However, a meta-analysis carried out by Jacob and DiazGranados (2013) established two separate break points for BMD and E-test, which are ≥1 mg/l and ≥1.5 mg/l, respectively.[19] Our study cannot comment on the latter break point criteria since we did not monitor treatment outcomes.

This highlights the importance of site-specific and continuous surveillance of MICs for guiding clinicians on the probable susceptibility of *S. aureus* to vancomycin in their patients and helping in empiric antibiotic selection against them.

Conclusion

Tests like BMD and E-test are carried out to determine the MIC which would give us an idea of the effectiveness of an antibiotic. However, these testing methods produce highly variable results. Observing the results obtained from our study and evaluating them along with the existing

literature, we would like to suggest that the E-test is a good alternative test method for detecting vancomycin MIC as it is less time consuming than BMD. Though the results are not the same in the two test methods, it gives an overall idea of the antibiotic which is effective or not for treatment. Additionally, since there is a significant difference between vancomycin MICs obtained by different methodologies, the susceptibility break points need to be adjusted accordingly. Clinical studies with a larger number of patients and including treatment outcomes are required to determine the relationship of MICs obtained using the two methods with vancomycin treatment failure.

Declarations

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