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Original Research Article

Detection of vancomycin susceptibility among methicillin resistant staphylococcus aureus in a tertiary care hospital

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ABSTRACT

Introduction: *Staphylococcus aureus* infections in current times have become challenging to treat because of advent of Methicillin Resistant *Staphylococcus aureus* (MRSA) strains which are concurrently resistant to a wide panel of drugs and posing a threat to clinicians and microbiologists globally. The optimal drug for treatment of such MRSA infections is vancomycin but strains with augmented Minimum Inhibitory concentration (MIC) for this drug also have surfaced.

Objectives: To know the frequency of MRSA isolates in various clinical samples with their antimicrobial sensitivity patterns and to equate agar dilution and E-test methods for MIC determination of vancomycin to MRSA strains.

Materials and Methods: A total of 50 non repeat clinical isolates of *staphylococcus aureus* isolates were collected from various clinical specimens and were tested for methicillin resistance using the cefoxitin disc diffusion test (30μg). All MRSA isolates were tested for specific MIC by agar dilution and E-test methods.

Results: 29 (58%) isolates were resistant to cefoxitin (MRSA). 13.8% isolates had MIC of 4μg/ml for vancomycin (VISA) by both agar dilution and E-test methods. However by agar dilution method 25 (86.2%) isolates exhibited vancomycin MIC of $\leq$ 2 μg/ml and by E-test 68.9% of the isolates showed MIC $\leq$ 2 μg/ml.

Conclusion: Multidrug resistant MRSA strains are on the rise and alternate drug of choice for these infections; vancomycin also is showing increased MIC so prudent use of this drug is advocated. E-test can detect MRSA strains with intermediate MIC values useful for detection of MIC creep so that vancomycin can be used rationally.

1. Introduction

Staphylococcus aureus has become a huge concern because of its high morbidity, high mortality attributes and both community-acquired and nosocomial infections are associated with it.[1] The advent of methicillin-resistant *Staphylococcus aureus* (MRSA) strains has further made it challenging to treat *Staphylococcus aureus* infections. The only therapeutic alternatives in many cases are limited to glycopeptides such as vancomycin or teicoplanin. However, in recent times treatment failures for even vancomycin have been reported. Now there is an increasing body of evidence which suggests that a relationship exists between vancomycin MIC and clinical vancomycin failure, despite the fact that in vitro MRSA strains are absolutely susceptible (MIC 2 g/mL) to vancomycin.[2] Although majority of these strains have a vancomycin MIC within the susceptible limits, yet a gradual and progressive upsurge in vancomycin MIC which is also acknowledged as the "MIC creep" has been on the rise in recent years.[3] As a matter of fact, after arbitrating on the incessant rise in the cases of failed vancomycin therapy CLSI thereupon abridged the breakpoints of vancomycin from 4 mg/L to 2 mg/L for susceptible strains of Staphylococcus aureus

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and for the resistant strains from 32 mg/L to 16 mg/L. Infections with MRSA isolates that reveal MIC creep can lead to poor prognostic outcomes, deferred therapeutic responses, amplified relapse rates, protracted hospital stay with consequent increased hospitalization costs and greater mortality rates.[4]

This awareness of data of infections with MRSA strains that have increased vancomycin MICs can help in the early identification of patients who are at perils of vancomycin treatment failure so that alternate treatment options can be explored at the right time.[5] Therefore, the present study aims to approximate the vancomycin susceptibility patterns amongst Methicillin Resistant *Staphylococcus aureus* isolates in a tertiary care hospital.

2. Materials and Methods

The study was conducted in the Department of Microbiology & Immunology, at Shri Guru Ram Rai Institute of Medical and Health Sciences & affiliated Shri Mahant Indiresh Hospital, Dehradun from November 2016 to April 2017. The study was approved by the institutional research board and ethics committee. A total of 50 non repeat clinical isolates of *staphylococcus aureus* were collected from diverse clinical specimens like pus, wound swab, blood culture, sputum, Broncho-alveolar lavage, pleural fluid and urine. Various attributes like colony characteristics, microscopic morphology, and biochemical reactions were used for preliminary detection of *Staphylococcus aureus* as per the standard protocol. Automated method; VITEK-2 (Biomerieux) was used for both identification and for determining the antibiotic susceptibility patterns. However manual method i.e. disc diffusion method based on mec A mediated oxacillin resistance and 30 μg cefoxitin disk as a surrogate marker for oxacillin was also utilized for detecting MRSA strains. Reference *Staphylococcus aureus* strains; *Staphylococcus aureus* ATCC 25923 was used as negative and ATCC 43300 was used as positive control for quality maintenance and consistency of results. Isolates showing inhibition zone size $\geq$ 22 were considered as sensitive (MSSA) and $\leq$ 21 mm were considered as resistant (MRSA) as per CLSI criteria for zone diameter breakpoints of *Staphylococcus aureus* for cefoxitin.[6]

CLSI guidelines were used to determine MIC of vancomycin by agar dilution method and by E-test method. ATCC strains; 25923 and ATCC 700698 of *Staphylococcus aureus* were incorporated within in all the test plates for quality and standardization. The least concentration of vancomycin that inhibited the visible growth of bacteria was considered as MIC of drug for that organism. Interpretation of MIC of vancomycin was done as per the CLSI guidelines for both agar dilution method and E-test strip method. Vancomycin MIC of $\leq$ 2 μg/ml was considered as the breakpoint for vancomycin susceptible (VSSA) MRSA strains, 4-8 μg/ml for vancomycin intermediate (VISA) strains and $\geq$16 μg/ml for vancomycin resistant (VRSA) strains.[6]

3. Results

Over-all 50 *Staphylococcus aureus* strains were isolated from various clinical specimens of which pus specimens contributed for bulk of the isolates (78%). 29 (58%) isolates were resistant (zone diameter <21mm) to cefoxitin while 21 (42%) were sensitive (zone diameter >22 mm) to cefoxitin.

Maximum number of MRSA isolates were recovered from Pus (75.8%) followed by 6.9 % each from blood, suction tip and tissue (Table 1). Allocation of patients on the basis of different sites from where MRSA was isolated is given in (Table 2). Majority of these isolates were recovered from the surgery wards (31%) followed by patients admitted in orthopedic wards (24.1%). Maximum number of isolates showed increased resistance to amoxicillin/clavulanic acid (89.7%) (apart from penicillin and cefoxitin for which 100% resistance was seen) (Table 3). However, linezolid sensitivity was observed in all MRSA isolates.

By agar dilution method for determining vancomycin susceptibility among MRSA isolates, a total of 25 (86.2%) isolates were having MIC for vancomycin in the range of 0.5- 2 μg /ml ($\leq$ 2 μg/ml) i.e. VSSA. By E-test method method 68.9% of the isolates had MIC $\leq$ 2 μg/ml. 31% isolates reported a vancomycin MIC of >2 μg/ml but <4 μg/ml. 13.8% VISA isolates with MIC of 4μg/ml were observed by both the methods. (Table 4).

The concentrations of vancomycin that inhibited growth of 50% and 90% of the isolates were defined as MIC_{50} and MIC_{90} respectively. MIC_{50} and MIC_{90} of the study isolates were found to be 1 μg /ml and 4 μg /ml by both the methods respectively.

Table 1: Distribution of MRSA isolates according to specimens (n=29)

Clinical Specimen	MRSA	
	n	Percentage (%)
Pus	22	75.8%
Blood	2	6.9%
Suction Tip	2	6.9%
Tissue	2	6.9%
Urine	1	3.5%
Total	29	100%

4. Discussion

In the current study isolation of methicillin resistant *Staphylococcus aureus* was maximum from pus 22(75.8%) followed by 2(6.9%) from blood. Similar observations have been reported by Chaudhri CN et al in their study wherein maximum number of isolates were isolated from pus

Table 2: Distribution of MRSA isolates according to Location/ site (n=29)

Location/Site	MRSA	
	n	Percentage (%)
Surgery Ward	9	31%
Orthopaedic Ward	7	24.1%
OPD	5	17.2%
Medicine Ward	2	6.9%
Medicine HDU	2	6.9%
Dermatology	1	3.5%
Surgical ICU	2	6.9%
Gynaecology/ Obstetrics	1	3.5%
Total	29	100%

Table 3: Trends of antibiotic resistance among MRSA isolates (n=29)

Antibiotic	Sensitive		Intermediate		Resistant	
	n	Percentage %	n	Percentage %	n	Percentage %
Penicillin	0	0	0	0	29	100%
Cefoxitin	0	0	0	0	29	100%
Amoxycillin/Clavulanic acid	3	10.30%	0	0	26	89.70%
Ciprofloxacin	6	20.6%	3	10.3%	20	68.9%
Erythromycin	12	41.3%	0	0	17	58.6%
Trimethoprim/Sulfamethoxazole	10	34.4%	2	6.8%	17	58.6%
Clindamycin	16	55.1%	0	0	13	44.8%
Tetracycline	12	41.3%	6	20.6%	11	37.9%
Gentamycin	13	44.80%	6	20.70%	10	34.50%
Linezolid	29	100%	0	0	0	0

Table 4: Comparative analysis of Vancomycin MIC by agar dilution and E-test methods (n=29)

Vancomycin MIC μg/ml	Agar dilution	E-test
	n (%)	n (%)
0.25	-	-
0.5	3(10.3)	4(13.8)
0.75	-	3(10.3)
1	13(44.8)	8(27.6)
1.5	-	3(10.3)
2	9(31.0)	2(6.9)
2.5	-	2(6.9)
3	-	2(6.9)
3.5	-	1(3.4)
4	4(13.8)	4(13.8)
Total	29	29

(76.7%) followed by blood (3.9%) and also by Sreenivasulu Reddy P et al wherein most of the isolates were also isolated from pus (69%) followed by blood (9%).[7,8] Cutaneous and sub cutaneous infections caused by *Staphylococcus aureus* often manifest in the form of abscesses and are formed to restrain the focus of infection.[9] Maximum isolates were from the IPD (82.8%) whereas only 17.2% isolates were from OPD. This is in concurrence with Suryadevara VD who in her research work has reported 70% of the isolates from IPD and 30% isolates from OPD.[10] The reasons can be attributed to healthcare workers who are chronic carriers for these isolates, emergence of strains with amplified resistance sequences and extended hospital stay especially in ICUs.

58% isolates were resistant to cefoxitin which is in concurrence with other studies as by Sanjana et al in which the isolation rate of MRSA was 39.6%, Juayang et al (40.6%) and Arora et al (46%).[11–13] In a tertiary care center, the incidence of MRSA may be higher since the probability of the patient being put on antimicrobial drugs beforehand is quite elevated and thus onset of selective pressure may negotiate a critical role in acquisition of resistance to most of the frequently used drugs.[14]

100% resistance was seen for penicillin and cefoxitin and similar observations have also been reported by Ramakrishna N.[15] Frequently prescribed antibiotics such

as ciprofloxacin erythromycin, clindamycin, gentamicin, tetracycline and co-trimoxazole all showed increased levels of resistance in our study as has also been reported from many parts of India particularly in association with MRSA.[10] Linezolid is one of the well-recognized alternative drugs for vancomycin and can be safely used for cutaneous and sub cutaneous infections, pneumonia, urinary tract infections and bacteremia caused by MRSA strains.[7]

The current study shows that 68.9% of isolates had an MIC of 0.5 - 2μg/ml whereas 31% isolates had an MIC >2μg/ml by the E.-test method. Similar observations have been made by Sreenivasulu Reddy P et al wherein 82% isolates showed MIC between 0.5-2μg/ml and 17% of isolates had an MIC of >2μg/ml.[8] Tandel et al however reported 60.3% strains with an MIC of >2μg/ml.[16] By the agar dilution method 86.2% isolates demonstrated MIC of 0.5 - 2μg/ml. However, by both the methods 4 (13.8%) strains exhibited a vancomycin MIC of 4 μg/ml and were therefore categorized as vancomycin intermediate (VISA). Kumari J et al have also reported 4.1% isolates with MIC >2 μg/ml by both methods.[17] There are several researches that have established a correlation for MRSA strains with increased vancomycin MICs (>1 μg/ml) and still within the context of susceptibility ($\leq$ 2 μg/ml) but unfortunately with proven clinical letdowns.[18,19]

Determination of MIC of vancomycin by E-test method was constantly higher by a range of 0.5-1 μg/ml than that worked out by agar dilution method. Such high MICs by E test as equated to agar dilution method have also been reported from other studies.[16] The important contributing factor could be the variances in the concentration gradient of vancomycin prepared in these tests. In agar dilution method the concentration of vancomycin is prepared in doubling dilutions or in geometric progression, whereas dilution of vancomycin concentrations is used in arithmetic progression in the E-test method so that the MICs for intermediate concentrations of the drug can also be determined.[20]

Area under the vancomycin concentration curve-to-MIC ratio (AUC/MIC) is also a significant factor to establish the effectiveness of vancomycin therapy in MRSA infection therapeutics.[21] The odds of attaining this ratio is nearly 100% if MIC of vancomycin is $\leq$0.5 μg/ml; and the likelihood reduces to practically 0% if the MIC of vancomycin is 2 μg/ml.[22,23] MIC/AUC ratio can be considerably prejudiced by even a sole dilution alteration in the MIC which in turn can significantly influence the outcome of therapy.[21] If just one dilution difference is pertinent to envisage the clinical consequences of MRSA infections, then the MIC method is a vital component of this reckoning.[19]

5. Conclusion

For MRSA infections Vancomycin still is the drug of choice and therefore should be judiciously used in treatment of such resistant infections as also observed in our study that MRSA continue to be multi drug resistant leaving clinicians with limited therapeutic options. However, a silver lining in our study is that efficacy of Linezolid still incites hope for VISA and VRSA cases. MIC creep needs further evaluation to optimize treatment modalities hence early detection of such strains in high susceptibility range could minimize the risk of emergence of more aggressive VISA and VRSA infections. For determination of vancomycin MIC dilution methods still are the gold standard, yet E-tests could be considered to establish vancomycin MIC in the intermediary zones and for observing trivial MIC changes.

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8. Conflict of Interest

The authors declare they have no conflict of interest.

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