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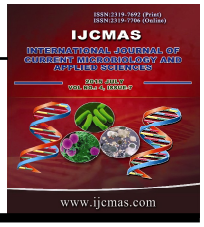

Original Research Article

Prevalence of Methicillin resistance in Staphylococcal blood isolates and correlation with Vancomycin MIC: A study from tertiary care hospital

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Keywords

Methicillin resistance in Staphylococcal blood, Vancomycin MIC

A B S T R A C T

Staphylococcus are the major cause of nosocomial and community acquired infections and has become increasingly prevalent worldwide. The prevalence however, varies markedly in hospitals in the same country, and from one country to another. Methicillin resistant is more common among these isolates. These strains are particularly important because they have limited therapeutic options. Glycopeptides such as vancomycin are frequently the antibiotics of choice for treating such infections. Given the importance of Staphylococci in hospital setting, the present study was done to find out the distribution of the various Staphylococcal species causing blood stream infections in this hospital and to analyze the prevalence of methicillin resistance among these isolates. MIC evaluation of vancomycin correlating it with resistance towards methicillin was done. Method: A total 128 Staphylococcus spp isolates consisting of 06 S. aureus and 122 coagulase negative staphylococci (CoNS) were isolated from blood specimens from various out patient departments and wards. The identification and the antimicrobial susceptibility testing (AST) were performed in the Vitek 2 compact automated system. Results : A total of 69 % (89/128 strains) were oxacillin resistant. In the present study, our hospital has a prevalence rate of 50 % of oxacillin resistant. S. aureus and 69% of oxacillin resistant other Staphylococci sps. We found three cases of Methicillin resistant Staphylococcus strains showing vancomycin resistance with MIC 32 μg/ml. All the three isolates were found to be resistant to several other antimicrobials. Conclusion: Our study demonstrates the high prevalence of methicillin resistance among hospital isolates of Staphylococci. Although vancomycin resistance was less frequent among these isolates, there is a definite shift towards higher values of MICs which might affect patient's clinical outcome. Indiscriminate use of vancomycin leading to the emergence and spread of vancomycin resistance in multidrug resistant strains is of growing concern in the recent years. Continuous efforts should be made to prevent the spread and emergence of vancomycin resistance by early detection of the resistant strains. Using proper infection control measures in the hospital setting and formation of antibiotic policy guide lines is highly recommended.

Introduction

Staphylococci are important causes of serious nosocomial and community-acquired infections and continue to cause a variety of clinical syndromes worldwide (1). In many hospitals and areas worldwide, the prevalence of Methicillin resistant Staphylococci (MRS) poses a serious therapeutic problem (2). The spread of these strains from the hospital to the community, coupled with the emergence of Vancomycin Intermediate Staphylococci(VIS) and Vancomycin Resistant Staphylococci,

(VRS) have become a major concern among healthcare providers (3).

MRS are notorious for their wide variations in antibiotic resistance patterns. They frequently develop chromosomal resistance to penicillins & cephalosporins. Due to the acquirement of the mecA gene they show resistance to a wide range of antibiotics which are commonly used in hospitals (4), including aminoglycosides, chloram-phenicol, macrolides, tetracycline fluro-quinolones, trimethoprim-sulfamethoxazole and clindamycin. (5,6).Glycopeptides are the only therapeutic alternative in many such cases and this represents an emergent challenge to the clinicians(2).

Trends towards increasing vancomycin Minimum Inhibitory concentration (MIC) though within susceptible range have been noted over the past few years (7). Years of exposure of strains to sub‑inhibitory concentrations of vancomycin is thought to be a key factor for increasing vancomycin MICs (8). Increase in the vancomycin usagecan create a selection pressure favoring the development of vancomycin resistant strains(9).Some studies report that sub optimal clinical outcomes are observed with vancomycin MICs between 1.5 and 2.0 g/ml, and recommend changing to alternate therapy (10, 11).

The emergence of decreased vancomycin susceptibility among staphylococci has led to evaluation of susceptibility tests performed by clinical laboratories to avoid imprecise vancomycin MIC determinations (11).Broth Microdilution method, the CLSI reference method for MICs determination method along with different phenotypic detection methods generate different values. (10). Given the importance of Staphylococci in hospital settings, this study was done to find out the distribution of the various Staphylococcal species causing blood stream infections in this hospital and to analyze the prevalence of methicillin resistance. Also since Minimum Inhibitory concentration (MIC) remains an important determinant in choosing the right antibiotics, MIC evaluation of vancomycin correlating it with resistance towards methicillin was done.

Materials and Methods

A total of 128 MRSA and MRCoNS isolated from blood samples, were collected and further analyzed.

Blood samples were collected from the patients of different inpatient and out patient departments of the Vydehi Institute of medical sciences & research center. This isa tertiary care teaching hospital, which serves the population of eastern region of Bangalore.

Blood cultures submitted to the microbiology laboratory were processed with the BacT/Alert® Plus (Biomerieux, Inc. Durham, NC 27704). Bacterial isolates processed according to standard microbiologic procedures. If gram-positive cocci in clusters were seen on a Gram stain, the blood culture was subcultured onto 5% sheep blood agar plates. The agar plates were incubated at 35^{o}C for 18-24 hours in aerobic atmosphere. Staphylococcus was identified by colony morphology, Gram staining & performing routine biochemicals like catalase & tube coagulase tests. The subsequent speciation and antimicrobial susceptibility testing (AST)was performed using the GPID and GP AST P628 cards of the Vitek 2 compact automated system (Biomerieux, Inc. Durham, NC 27704). Results yielding a quality of identification of 90% or higher were accepted. The cardinoculation, and reading results were performed according to the instructions of the manufacturer.

For oxacillin, the CLSI breakpoints used were as follows: *S.aureus* and Staphylococcus lugdunensis (susceptible MIC of _2 mg/L; resistant MIC of _4 mg/L), Staphylococcus epidermidis (susceptible MIC of _0.25 mg/L; resistant MIC of _0.5 mg/L) and coagulase-negative staphylococci other than S. epidermidis (susceptible MIC of _0.25 mg/L; intermediate MICof 0.5–2.0 mg/L; resistant MIC of _4 mg/L).

Results and Discussion

The analyzed 960 blood samples, microbiological growth were seen in 187 (19.4%) samples. Among these 128 (63%) yielded Staphylococcus spp. The overall prevalence of Staphylococcal strains was 68%.

According to Vitek2 compact identification, of the total 128 blood isolates of Staphylococci sps, S. hominis was the commonest species (62/128, 48.4 %) followed by *S. haemolyticus* (38/128, 30%), S. epidermidis (18/128, 14 %), *S.aureus*(6/128, 5%), S.warneri (3/128, 2.3 %) and one isolate of S.capitis (1/128, 0.78 %). The prevalence of methicillin resistance among Staphylococcal strains isreported in table 1.

A total of 69 % (89/128 strains) were oxacillin resistant of which 84.2 % (32/38) *S. haemolyticus* strains, followed by *S.epidermidis* 78% (14/18), S.hominis61.2% (38/62), *S.aureus*50% (3/6), S.warneri33.3% (1/3) & none of the *S.capitis* isolates were resistant to oxacillin.

As revealed in the present study, our hospital has a prevalence rate of 50 % of oxacillin resistant. *S.aureus* and 69% of oxacillin resistant other Staphylococci sps.
The vancomycin MIC distributions among 128 strains methicillin resistant Staphylococci are reported in figure 1. A total of 80strains had MIC 1µg/ml, followed by 30 strains MIC 0.5µg/ml and the least were 15 strains with MIC 2 µg/ml.

We found three cases of Methicillin resistant Staphylococcus strains showing vancomycin resistance with MIC 32 µg/ml. In first case, the isolatewas identified as S. epidermidis, from a new born baby who was admitted in Paediatric ward; in the second case the isolate was S. haemolyticus from a 26 years old patient admitted in MICU, and in the third case the isolate was again S. haemolyticus from a new born baby admitted in NICU of our hospital. All the three isolates were found to be resistant to several other antimicrobials such as penicillin, linezolidclindamycin, cipro-floxacin, erythromycin, teicoplanin, rifampin, and trimethoprim/sulfa-methoxazole apart from methicillin & vancomycin.

In our study we found no strains with low sensitivity to vancomycin (MIC between 4 and 8 µg/ml).

The prevalence of MRS strains varies globally. Methicillin resistance among staphylococci is widespread in India and ranges from 30-80% (12,13).

Within a country, there may be local variations in predominant hospital and community strains of MRS (CA-MRS). The resistance pattern of CA-MRS is essentially different from that of hospital acquired MRS (HA-MRS). Unlike CA-MRS, hospitals strains display more drug resistance in an attempt to survive in hospital environment. Most of these isolates appear to have developed from preexisting MRS infections(4).The resistance patterns are liable to continuous changes over a period of time, owing to changes in antibiotic prescription patterns due to awareness

among healthcare workers and infection control measures. The development of antibiotic resistance in developing countries like ours seems to be very much related to the irrational antibiotic usage due to it's easy availability at the drug store without prescription, injudicious use in hospitals and uncontrolled use in agriculture, animal husbandry and fisheries (9).

Vancomycin is the main antimicrobial agent available to treat serious infections with MRS but unfortunately, there is decrease in vancomycin susceptibility to these pathogens. Vancomycin-resistant strains are those for which the MICs are 16 μg/ml, and those with MICs 8 μg/ml are intermediate strains, hetero resistant strains are those for which the MICs are 1.5 to 4 μg/ml, and susceptible strains were those for which the MICs were 1 μg/ml (16). The isolation of vancomycin intermediate and resistant strains has recently been reported from many countries(3). Many reports from north India and south India (10,14) also recorded the emergence of low level and intermediate vancomycin resistance (15).

Considering that the treatment of staphylococcal infections depends on the methicillin resistance, and that this is habitually based upon the phenotypic detection methods, the inconsistency of results may have important implications on inadequate therapy (17). Automated instruments offer many advantages in clinical laboratories, especially in hospitals. Such instruments improve workflow and provide faster results than routine conventional phenotypical methods. Correct detection of methicillin resistance among these isolates in the clinical laboratory is important to guide therapy and to promote the correct use of glycopeptides (18).

In automated Vitek 2 system, detection of methicillin resistance was based solely on determination of oxacillin MIC. These automated tests provide results in about 10 has compared to more conventional phenotypic methods which can take up to 24 h. This may have a potential impact on the optimal management of these infections. This system detects metabolic changes by the results of biochemical tests and photometric determination of growth and calculates MICs using a unique algorithm (8)

Molecular methods were recently introduced to improve results, but for most of the clinical laboratories these techniques are still too expensive(17). It is important to point out that mortality rates in non-treated patients with bacteremia are greater than those of patients receiving adequate antimicrobial therapy and that false positive resistance accounts for increased of costs, prolonged stay in hospitals(12), further clinical work, request of more cultures, and unnecessary use of antibiotics such as vancomycin.(19,17)

In view of the limited therapeutic options for the treatment of MRS infections, judicious use of vancomycin, continuous surveillance for VIS and VRS strains, and appropriate infection control practices like a regular surveillance of hospital associated infection, monitoring of antibiotic susceptibility pattern and formulation of definite antibiotic policy may be helpful for the prevention of spread of such strains in the hospital environment (20). Moreover, in our country nationwide surveillance program should be carried out to map the vancomycin susceptibility pattern(9). Decreasing the antibiotic pressure is necessary to control the emergence of resistant strains in the hospital and in the community

Table.1 Prevalence of Methicillin resistance of Staphylocci isolates

	Total no strains n=128	%
Staphylococcus hominis	59	47.2
Staphylococcus haemolyticus	38	30.4
Staphylococcus epidermidis	17	13.6
Staphylococcus aureus	06	4.8
Staphylococcus warneri	02	1.6
Staphylococcus auricularis	01	0.8
Staphylococcus capitis	01	0.8
Staphylococcus sps	01	0.8

Table.2 MIC of vancomycin for methicillin resistant Staphylocci isolates

MIC value of vancomycin mcg/ml	Total no strains n=128	%
0.5	30	23.4
0.5-1	0	-
1	80	62.5
1-2	0	-
2	15	12
4	0	-
8	0	-
>32	3	2.3

The difference in Methicillin resistant prevalence found in different studies compared to our study may be due to several factors the length of study period, number of study sites and the sample size. A larger sample size would also have had a greater probability for increased number of observations. This was restricted due to limited resources and time.

In conclusion, this study indicated the magnitude of antibiotic resistance in and around the study area. The study confirms the high circulation of methicillin resistant Staphylococci. The prevalence of methicillin resistance among CONS was more compared to *S.aureus*. If treatment with vancomycin is continued it could result in clinical failure as there is definite shift towards higher values of MICs. This study also emphasizes the need for continuous monitoring of MIC levels of vancomycin in Methicillin resistant strains and it is essential that clinicians take the MIC testing method into consideration when choosing antimicrobial chemotherapy. Lastly appropriate care should be taken in the clinical microbiology laboratory while detecting the methicillin and vancomycin resistance, as there is only limited therapeutic alternatives available to treat these infections.

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