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Original Research Article

Antibiotic susceptibility pattern of *Staphylococcus aureus* in tertiary care hospital, SRMSIMS, Bareilly, U.P.

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ABSTRACT

Background: *S. aureus* has been recognized as continuously challenging the clinicians despite the availability of antibiotics from nearly 70 yrs and emergence of various types of antibiotic resistance mechanisms especially to methicillin and vancomycin, which was the theme of this study. The study was to determine the antibiotic susceptibility of *Staphylococcus aureus* in Tertiary Care Hospital, SRMSIMS, Bareilly and also to determine the current status of Vancomycin susceptibility in our Hospital setup considering E-Test as gold standard.
Methods: This study was prospective in design from 1st January 2014 to 31st December 2014 and conducted in the Department of Microbiology, SRMSIMS, Bareilly. All *S. aureus* strains were screened for vancomycin resistance by Dual strip E- test.
Results: Out of 505 *Staphylococcus aureus* isolates, we found that MRSA, VISA, and VRSA as 80.8%, 0.6% and 0.0% respectively. In cases of MRSA, antibiogram showed sensitivity in the range of 99.4 -100% for glycopeptides, and sensitivity to levofloxacin, chloramphenicol was 77.5% and 65.2% respectively.
Conclusions: On evaluation of MIC by E-strips, we found that 17% of MRSA were showing MIC values quite near to the MIC value of VISA. So, these strains may convert to VISA, if the injudicious use of vancomycin antibiotics is not stopped.

Keywords: *S. aureus*, MSSA, MRSA, VISA, VRSA

INTRODUCTION

Staphylococcus aureus is the most clinically significant species of *Staphylococci* has been recognized as an important cause of human disease for more than 100 years.[1] It is one of the pathogens of greatest concern because of its intrinsic virulence factors, its ability to cause diverse array of life threatening infections, it's competency to adapt to different environmental conditions and its nasal carriage, which accounts for possible spread and re infection.[2] It is one among the top three major potential pathogens responsible for community and hospital acquired infections causing diseases ranging from relatively minor skin and soft tissue infections primarily to life-threatening systemic infections which can be either toxin/non-toxin mediated, leading to high morbidity and mortality throughout the world.[3,4]

Infections by *S. aureus* are continuously challenging the clinicians despite the availability of antibiotics from nearly 70 yrs. This was due to the emergence of various types of antibiotic resistance mechanisms especially to methicillin and vancomycin, which was the theme of several epidemiological studies.[5,6] The rate of nosocomial MRSA approximately doubled from 30% in 1990s to

80% in current scenario for many countries including India.[7] The incidence of MRSA varies from 25% in Western India to 50% in South India.[8] The increasing prevalence of MRSA resulted in over utilization of vancomycin as first-line glycopeptides due to decreased susceptibility or increase MIC values to Vancomycin and this inturn lead to totally resistant of *S. aureus* to vancomycin.[9,10] Objective of the present study was undertaken to determine the antibiotic susceptibility of *S. aureus* in Tertiary Care Hospital, SRMSIMS, Bareilly and also to determine the current status of Vancomycin susceptibility in our Hospital setup considering E-Test as gold standard.

METHODS

This study was prospective in design of complete one year from 1st January 2014 to 31st December 2014 and conducted in the Department of Microbiology, SRMSIMS, Bareilly. Out of 2639 samples, 505 were isolated as *S. aureus* from various clinical specimens like that pus, wound or vaginal swabs, blood, body fluids (CSF, pleural fluid, ascitic fluid) urine, sputum, endotracheal secretion etc. were included from both IPD & OPD. Institutional Ethical clearance was obtained.

Streak culture method was employed for sample by inoculation on Blood agar (HiMedia M073) and MacConkey's agar (HiMedia M082) after receiving samples. Culture plates were incubated at 37^0C aerobically for 24-48 hours. Plates were observed for typical colony characteristics of Staphylococcus aureus on Blood agar (β-hemolysis). Gram's staining was performed, and observed for GPC in clusters, under oil immersion lens of microscope. *S. aureus* was confirmed by Catalase test (3% H_2O_2), slide and tube coagulase test and Antibiotic susceptibility testing (AST) was done on Muller Hinton Agar (HiMedia M173) by Modified Kirby-Bauer disc diffusion method by using McFarland standard inoculums and diameter of zone of inhibition was measured and interpreted according to CLSI guidelines 2007 (32). *S. aureus* ATCC 25923 were used as a vancomycin susceptible control strains and Enterococcus faecalis ATCC 51299 as vancomycin resistant control strain. Methicillin and Vanomycin susceptibility were also tested, using Cefoxitin disc (30 µgm) (HiMedia) and Vanomycin disc (10 µgm) (HiMedia) along with routine AST. MIC of Cefoxitin and Vancomycin for each isolate was determined and recorded by Dual strip Epsilometer test (E-test) [HiMedia Ezy MIC™ EM077 (VAN: 0.19-16.0 mcg/ml, CX: 0.5-64 mcg/ml)]. All other antibiotic discs were procured from Hi Media Laboratories Ltd only.

Statistical analysis

Data collected were cleaned, filled in the excel sheet and analyzed. Percentages and proportions were used to express data.

Inclusion criteria

All *S. aureus* strains collected from various clinical specimens will be screened for vancomycin resistance.

Exclusion criteria

Cases of wound infection which did not yield the growth of *staphylococci*, but yielded growth of other bacteria, fungal, commensal growth and mixed infection.

RESULTS

Out of 2639 clinical specimens, only Gram positive cocci were observed in 1389 (52.6%) samples followed by 685 (25.9%) samples were shown both *Gram negative bacilli* and *Gram positive cocci*, 152 (5.8%) samples fungal growth, 230 (8.7%) samples commensal growth & 183 (6.9%) samples had shown no growth of any bacteria. In these 183 cases, there was no visible discharge or collection but a clinical suspicion of wound infection was made clinically because of their non-healing nature. Out of 1389 samples had shown GPC, 505 were confirmed as *S. aureus* by above mentioned characteristic biochemical tests. Our study were shown MSSA 18.6%, MRSA 80.8%, VISA 0.6% and VRSA 0.0% whereas rest of 884 gram positive isolates as commensal/contaminant flora like Micrococci and Coagulase negative staphylococci (CoNS) that has been excluded from study.

On analysis of antibiogram

Out of three groups of MSSA, MRSA and VISA found that MSSA was shown sensitivity for most of the applied antibiotics followed by MRSA which is comparatively more resistant than MSSA for most of the antibiotics while VISA is highly resistant pathogen which has been shown in Table 1 and 2. Sensitivity for various groups of antibiotics as shown in table-1, MSSA shown almost 100% sensitivity for glycopeptides, followed by Levofloxacin (94.4%), Chloramphenicol, Cotrimoxazole, Tetracycline which shown sensitivity in between 80-90% approx. As far as Macrolides were concerned, these shown sensitivity in range of 73% while least sensitivity was recorded with beta-lactam antibiotics that was shown only 14% sensitivity. On analysis of uropathogens, all pathogens had shown 100% sensitivity to urinary antiseptics like Nitrofurantoin and Norfloxacin. As far as the sensitivity pattern of MRSA is concerned, it showed sensitivity in the range of 99.4 -100% for glycopeptides while sensitivity to Levofloxacin, Chloramphenicol was 77.5% and 65.2% respectively, followed by Aminoglycosides, Macrolides, Cotrimoxazole and Tetracycline that was found to be in the range of 5.6-28.7% and lastly they were found least sensitive 4.2% to beta-lactam antibiotics. Urinary isolates showed 68.9% and 47.2% sensitivity to Nitrofurantoin and Norfloxacin respectively and found 100% resistant to Cefoxitin hence all isolates were MRSA.

Table 1: Antibiotics susceptibility pattern of MSSA and MRSA.

S. no	Drugs	MSSA						Total	MRSA						Total
		S	%	IS	%	R	%		S	%	IS	%	R	%	
1.	*P	10	14.0	0	0.0	61	86.0	71	14	4.2	0	0.0	320	95.8	334
2.	*Cx	71	100.0	0	0.0	0	0.0	71	0	0.0	0	0.0	334	100.0	334
3.	*LE	67	94.4	3	4.2	1	1.4	71	259	77.5	29	8.7	46	13.8	334
4.	*G	70	98.6	1	1.4	0	0.0	71	19	5.6	63	18.8	252	75.4	334
5.	*C	64	90.1	5	7.0	2	2.9	71	218	65.2	60	18.1	56	16.7	334
6.	*E	52	73.2	9	12.7	10	14.1	71	22	9.6	53	15.8	249	74.6	334
7.	*CD	61	86.0	4	5.6	6	8.4	71	61	18.3	21	6.2	252	75.5	334
8.	*TEI	70	98.6	1	1.4	0	0.0	71	332	99.4	2	0.6	0	0.0	334
9.	*VA	71	100.0	0	0.0	0	0.0	71	334	100.0	0	0.0	0	0.0	334
10.	*LZ	71	100.0	0	0.0	0	0.0	71	332	99.4	1	0.3	1	0.3	334
11.	*TET	65	91.6	5	7.0	1	1.4	71	96	28.7	48	14.5	190	56.8	334
12.	*COT	60	84.5	6	8.5	5	7.0	71	61	18.2	42	12.5	231	69.3	334
13.	*NIT	23	100.0	0	0.0	0	0.0	23	51	68.9	4	5.4	19	25.7	74
14.	*NX	23	100.0	0	0.0	0	0.0	23	35	47.2	7	9.6	32	43.2	74

*P=Penicillin; Cx=Cefoxitin; LE=Levofloxacin; G=Gentamicin; C=Chloramphenicol; E=Erythromycin; CD=Clindamycin; TEI=Teicoplanin; Lz=Linezolid; VA=Vancomycin; TET=Tetracycline; COT= Cotrimoxazole; NIT= Nitrofurantoin; NX=Norfloxacin.

Table 2: Antibiotics susceptibility biogram of VISA.

S. no	Drugs	VISA						Total
		S	%	IS	%	R	%	
1.	*P	0	0.0	0	0.0	3	100.0	3
2.	*CX	0	0.0	0	0.0	3	100.0	3
3.	*LE	2	66.7	0	0.0	1	33.3	3
4.	*G	1	33.3	0	0.0	2	66.7	3
5.	*C	0	0.0	0	0.0	3	100.0	3
6.	*E	0	0.0	0	0.0	3	100.0	3
7.	*Cd	2	66.7	0	0.0	1	33.3	3
8.	*TEI	3	100.0	0	0.0	0	0.0	3
9.	*VA	0	0.0	3	100.0	0	0.0	3
10.	*LZ	3	100.0	0	0.0	0	0.0	3
11.	*TET	2	66.7	0	0.0	1	33.3	3
12.	*COT	0	0.0	0	0.0	3	100.0	3
13.	*NIT	0	0.0	0	0.0	0	0.0	0
14.	*NX	0	0.0	0	0.0	0	0.0	0

Table 3: Interpretation of MIC of vancomycin using E- strips.

Vancomycin	Mic (mcg/ml)	No. of patients	Percent
VSSA*	< 0.5	100	19.9
	0.5 – 1	69	13.7
	1 – 1.5	106	21.2
	1.5 – 2	227	45.0
VISA*	4-8	3	0.6
VRSA*	>16	0	0.0
Total		505	100.0

*(According to manufacturer guidelines Cefoxitin MIC value <6 and >6 mcg/ml, are considered as MSSA and MRSA respectively, Vancomycin MIC value <2, 4-8, >16 mcg/ml, are considered as VSSA, VISA, VRSA respectively.)

To comment on antibiotic susceptibility pattern of VISA as shown in Table 2 is not justifiable because of mere number of isolation of VISA in this study and need higher number of VISA isolates for description. But our study had shown that these VISA strains were found to be 100% resistant to Beta- lactam, Chloramphenicol, Macrolides and Co-trimoxazole antibiotics whereas 100% sensitivity shown to linezolid, Teicoplanin

antibiotics and rest antibiotics like Levofloxacin, Gentamicin, Clindamycin and Tetracycline had shown sensitivity in the range between 33% - 66%. Since in our study we did not find any VRSA strains and cannot comment on the antibiogram of such isolates.

Interpretation of MIC by E- test

Our study indicates that out of 505 *S. aureus* strains, 411 strains were isolated as MRSA had Cefoxitin MIC value >6 mcg/ml & rest 94 were identified as MSSA (Cefoxitin MIC <6 mcg/ml). On evaluation of MIC values of Vancomycin found that out of 411 MRSA isolates 408 were fall in category of VSSA as their MIC value is <2 µg/ml which is the indicator value to label a strain as VSSA. *S. aureus* strains were observed the MIC value for Vancomycin in between 4-8 µg/ml which is the indicator value to label to the isolate as VISA and our study has isolated 3 VISA strains. Almost half of VSSA strains were quite near to the higher range of MIC of VSSA or near to the range of MIC of VISA which has been shown in Table 3.

DISCUSSION

S. aureus is a major human pathogen and is one of the commonest causative agent of Community and Hospital acquired infections.[4] The treatment of *S. aureus* infection has become problematic because of emergence of resistance to Penicillin, Methicillin, Vancomycin and many other antibiotics, by acquiring several resistance mechanisms. Increased antimicrobial resistance for such an organism is, therefore a cause of concern. In the past few decades MRSA has emerged as an important nosocomial pathogen worldwide. A multicentric study had conducted in India involving 17 tertiary care Hospitals reported 41% prevalence of MRSA and other studies had shown in India the prevalence of MRSA ranging from 54.8% Anupurba et al to 80.89% Verma, et al.[11-13] In our study, 80.8% isolates turned out to be MRSA from a total of 505 S. aureus strains. The higher rate in our study may be attributed to the fact that the study was conducted at a tertiary care multispecialty centre with more patients coming from periphery and small nursing homes, where injurious use of antibiotics and inadequate infection control policies are prevalent. Prolonged or broad-spectrum antibiotic therapy predisposes patients to infections with antibiotic-resistant organisms like MRSA Hartemann-Heurtier et al.[14] Increasing prevalence of MRSA, lead to the extensive use of Vancomycin. This inturn lead to the decreased susceptibility to Vancomycin all over the World including India, this was soon followed by strains of *S. aureus* that were totally resistant to vancomycin.[10,15] Such resistance resulted in serious clinical and public health consequences because currently a very few licensed alternatives are available to treat vancomycin resistant S. aureus infections.[16]

On analysis of Antibiotic susceptibility Pattern the resistance of MRSA to a wide range of antibacterials is well documented. The antibiotic sensitivity results showed that all MRSA isolates were significantly more resistant to antibiotics than MSSA. The resistance of MRSA to β lactams like penicillin was 95.8% in the present study. Similar findings were seen in the studies by Anupurba et al, Gupta et al, Uma choudhary et al and Anvikar et al.[12,17-19] 75.41% resistance of MRSA isolates to gentamicin was observed as compared to Majumder et al 70.3%, Hanumanthappa et al 81.39%, Pulimood et al 85.5%.[20-22] For years, Macrolides have been used as an alternative to penicillin and cephalosporins in the treatment of infections caused by gram positive bacteria, but the development of macrolide resistance has now limited the use of these antibiotics. In the present study, 74.6% of MRSA isolates were resistant to erythromycin similarly higher rate was observed in studies of Gupta et al 100%, Anvikar et al 95.9% and Hanumanthappa et al 93.02%.[17,19,21] In present study 75.5% of MRSA isolates were resistant to clindamycin by disc diffusion. A low percentage of 6.3% was reported by Choudhary et al and 30% Thouverez et al they have opined that clindamycin can be used as first line agent for treatment of MRSA infections.[18,23] Clinical isolates of constitutive MLS resistant staphylococci are continuing to increase in frequency and this trend may be a reflection of the increased clinical use of Clindamycin. Linezolid, the oxazolidinone has shown 99.4% efficacy and 0.3% resistant pattern and similar consistent activity of linezolid against MRSA has also been shown by Stevens et al, however linezolid resistance in *S. aureus* has been reported by Tsiodras et al.[24,25] As linezolid's antibacterial activity is comparable with that of vancomycin in the present study and can be used as an alternative to vancomycin in treating MRSA infections. MRSA is of serious therapeutic concern not only due to its resistance to Methicillin, but also because of resistance to many other antimicrobials that are used on regular basis in Hospitals. Therefore, the most reliable and sustained therapeutic agent against methicillin-resistant *S. aureus* (MRSA) strains is Vancomycin.[26] There is still controversy in clinicians regarding the outcome of vancomycin treatment in MRSA.

In the present study, vancomycin susceptibility was detected by both disc diffusion and E-strip method. The testing of vancomycin resistance in *S. aureus* has been a challenge in clinical laboratory, as presently MIC value determination by E-Test are only considered gold standard for determining vancomycin susceptibility.[27-29] Several authors have postulated that higher MIC values provided by the E-Test is more reliable in predicting vancomycin treatment response.[30] CDC has approved E-test for detection of vancomycin resistance.[29] As routine use of agar dilution is cumbersome and labour intensive. Also E-test method has the advantage of being easy to perform as a disc diffusion test, cost-effective for testing only one drug for one strain and interpretation of results is also easy.[24] Almost All strains showed sensitive zone

in disc diffusion testing and the MIC of all strains was <4 µg/ml. Only 3 isolates [0.6%] of *S. aureus* showed the MIC range between 4-8 µg/ml, they were termed as VISA. Widespread use of vancomycin to treat infections caused by MRSA has been reported to result in the emergence of low level resistance. VISA strains have been reported by Tenover et al in New York and Hiramatsu et al in Japan, at present the proportion of MRSA with reduced susceptibility to vancomycin is well known.[27,31] VRSA and VISA isolates have been reported by several researchers like Thati, Tiwari, Saha et al, and Menezes et al who stated that it was mainly due to excessive use of antibiotics in intensive care units and in other health care sectors.[9,10,32,33] Anupurba et al, Uma choudhary et al, Hanumanthappa et al, Pulimood et al, Thouverez et al, Kakru et Al, Vidhani et al, Roveta et al, Siddiqi et al and Mehta et al reported 100% percent susceptibility to vancomycin.[12,18,21-23,34-38] Since in our study we did not find any VRSA strains, we cannot comment on the antibiogram of such isolates, but various studies across the country have reported antibiogram of VRSA strains.

Failure with vancomycin occurs due to its slow bactericidal activity, low penetration in tissues and its increasing MICs. The increase in vancomycin MIC could lead to the increase in the frequency of hetero‑resistant VISA. Subpopulations of MRSA strains may have VISA selected by vancomycin treatment. Furthermore, increased vancomycin MIC has been correlated with adverse clinical outcomes in some studies.[30,39] Therefore alternative therapies should be employed where vancomycin MIC is >1 µgm/ml to avoid treatment failure. Antimicrobial agents effective against VISA and VRSA includes linezolid, daptomycin, tigecycline, quinupristin/dalfopristin, and also in vitro activity against VISA and VRSA have been demonstrated by dalbavancin, telavancin, oritavancin, ceftobiprole, and iclaprim. The emergence of VRSA is a critical concern to the therapeutic dilemma caused by the presence of multi drug resistant organisms in recent years.[32] Isolates of vancomycin resistant *S. aureus* have emerged in many parts of the world. These isolates appear to achieve clinically relevant levels of resistance to vancomycin that leads to treatment failure. This also necessitates to find out better treatment policies and also to use cheaper and effective alternative anti-MRSA drugs so as to reduce the antibiotic pressure on vancomycin. Also clinicians should continue to exercise caution in their use of vancomycin in order to preserve this useful antibiotic and prolong its therapeutic usefulness.

CONCLUSION

So lastly it can be concluded that the continuous misuse of antibiotics may turn those MRSA strains that have higher MIC, as VISA strains. Hence based on the above observations, determination of MIC values for all *S. aureus* isolates prior to administration of vancomycin is necessary in order to avoid emergence of resistant isolates in future. In the emergent conditions, the clinicians can do start the treatment of patients with the best possible antibiotics for the *S. aureus* like vancomycin, teicoplanin etc. but as soon as antibiotic sensitivity reports arrived, if we found the same sensitivity pattern, continue the same therapy till the acute stage, otherwise patient should be de-escalated for some other antibiotics, to avoid the misuse of antibiotics & development of resistant strains. As current study only indicates the tip of iceberg and more studies should be undertaken in future to monitor the emergence of resistance to these antibiotics.

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