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Vancomycin Resistance in Clinical Isolates of Methicillin Resistant *Staphylococcus aureus* from a Tertiary Care Hospital

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Abstract: *Staphylococcus aureus* is one of the most common causes of Blood Stream infections (BSI), skin and wound infections, osteomyelitis, endocarditis, and nosocomial infections, especially pneumonia, surgical site infections (SSI), and continue to be a major cause of community-acquired infections. Methicillin Resistant *Staphylococcus aureus* (MRSA) is an important cause of community and hospital acquired infections. MRSA are mainly nosocomial and are increasingly reported from many countries worldwide. The purpose of present study was to determine the sensitivity of *S. aureus* isolated from infected patients to methicillin and to evaluate the possible presence of VRSA in our tertiary care hospital. Staphylococci were isolated and identified by standard microbiological procedures. Methicillin resistance was detected by using cefoxitin (30 µgm) by disc diffusion method. MRSA strains detected were then subjected to vancomycin agar screen test and E test to detect vancomycin resistance. Out of the total 500 *S.aureus* isolates, methicillin resistance was observed in 47.4% of isolates**.** . By E-test, 24.1 % MRSA isolates had Vancomycin MIC value of 0.75 and 21.9% of MRSA had Vancomycin MIC value of 0.5. Only one isolate had MIC value of 2.

Keywords: S.aureus, MRSA, Vancomycin, E-test.

INTRODUCTION

Staphylococcus aureus is one of the most common causes of Blood Stream infections (BSI), skin and wound infections, osteomyelitis, endocarditis, and nosocomial infections, especially pneumonia, surgical site infections (SSI), and continue to be a major cause of community-acquired infections[1,2].

Several epidemiologic studies have demonstrated that infections due to *S. aureus* are associated with increased burden on healthcare resources and increased morbidity and mortality [3]. The development of antimicrobial resistance has been regarded as a consequence of their use with their introduction nearly 70 years ago and is continuously worsening [4].

Methicillin Resistant *Staphylococcus aureus* (MRSA) is an important cause of community and hospital acquired infections. MRSA are mainly nosocomial and are increasingly reported from many countries worldwide [5]. In 1980s, because of widespread occurrence of MRSA, empiric therapy for Staphylococcal infections (particularly nosocomial sepsis) was changed to vancomycin in many health care institutions. Vancomycin use in United States increased during this period because of the growing numbers of infections with *Clostridium difficle* and coagulase negative Staphylococci (CoNS) in health care institutions. Thus, the early 1990s have shown a significant increase in vancomycin use. As a consequence, selective pressure was established that led to emergence of strains of *S. aureus* and other species of Staphylococci with decreased susceptibility to vancomycin and other glycopeptides[6].

There are different breakpoints used in defining vancomycin susceptibilities in different countries. This has led to confusion in the definitions and clinical significance of vancomycin resistance. According to theClinical and Laboratory Standards Institute (CLSI)[7]. Staphylococci for which MIC of vancomycin is ≤ 2 µg/mL are sensitive, while isolates for which MIC of vancomycin is 4-8 µg/mL are defined as intermediate sensitive (vancomycin-intermediate *S. aureus*, VISA). Strains having MIC of vancomycin ≥ 16 µg/mL are designated resistant (vancomycin-resistant *S. aureus*, VRSA). Though there have been only a few reports of VRSA, the high prevalence of MRSA and vancomycin use, both thought to be risk factors for VRSA, make the widespread dissemination of these organisms an alarmingly realistic possibility. Such resistance could result in serious clinical and public health consequences because, currently, few licensed

alternatives to vancomycin are available to treat serious resistant *S. aureus* infections.

The purpose of present study was to determine the sensitivity of *S. aureus* isolated from infected patients to methicillin and to evaluate the possible presence of VRSA in our tertiary care hospital.

MATERIALS AND METHODS

This prospective study was conducted in the Department of Microbiology, Pt. B.D. Sharma University of Health Sciences, Rohtak. A total of 500 clinical isolates of *Staphylococcus aureus* were isolated from various clinical specimens, collected from patients irrespective of age and sex, were included in present study.

Staphylococci were isolated and identified by standard microbiological procedures [8]. Antimicrobial susceptibility testing was performed using Kirby Bauer disc diffusion method [9] on Mueller Hinton agar using the criteria of standard zone sizes of inhibition to define sensitivity or resistance to different antimicrobial agents according to the recommendations of the Clinical and laboratory Standard Institute (CLSI 2017)[7]. Methicillin resistance was detected by using cefoxitin (30 µgm) by disc diffusion method. MRSA strains detected were then subjected to vancomycin agar screen test and E test to detect vancomycin resistance [7].

RESULTS

Out of the total 500 *S. aureus* isolates, methicillin resistance was observed in 47.4% of isolates. Table 1 shows that number of MRSA isolates was maximum from pus and pus swabs (56.5%), followed by blood (36.9%) and least from throat swabs (zero %). All MRSA Isolates were resistant to penicillin. MRSA showed resistance to amoxicillin/clavulinic acid (92.4%), chloramphenicol (64.6%), ciprofloxacin (51.1%), cotrimoxazole (51.1%). Resistance to linezolid was only 0.8%. All methicillin sensitive *S. aureus* (MSSA) isolates were resistant to penicillin. MSSA showed resistance to ciprofloxacin (43.7%), amoxicillin/clavulinic acid (28.5%)(Table 2). All the MRSA isolates were also subjected to Vancomycin agar screen test but no growth was shown by any isolate. This shows that all the MRSA strains were sensitive to vancomycin. By E-test, 24.1 % MRSA isolates had vancomycin MIC value of 0.75 and 21.9% of MRSA had vancomycin MIC value of 0.5. Only one isolate had MIC value of 2 (Table 3).

Table-1: Distribution of staphylococcal isolates among various clinical specimens

Samples	Number of MRSA isolates(n=232)	Total number of *S. aureus* isolates(n=500)	Percentage
Pus	182	322	56.5
Urine	22	70	31.4
Blood	24	65	36.9
Sputum	4	15	26.7
CSF	2	10	20
HVS	3	15	20
Throat swab	0	3	0

Table-2: Antibiotic resistance pattern of mrsa and mssa

Drugs	MRSA [n=237]		MSSA[n=263]	
	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)
Penicillin	0(0)	237(100)	0	263(100)
Cefoxitin	0(0)	237(100)	263(100)	0(0)
Amoxicillin/ clavulinic acid	18(7.6)	219(92.4)	188(71.5)	75(28.5)
Erythromycin	132(55.7)	105(44.3)	221(84)	42(16)
Doxycycline	179(75.5)	58(24.5)	247(93.9)	16(6.1)
Ciprofloxacin	116(48.9)	121(51.1)	148(56.3)	115(43.7)
Nitrofurantoin	19(86.4%)	3(13.6%)	43(89.6%)	5(10.4%)
Linezolid	235(99.2)	2(0.8)	263(100)	0(0)
Trimethoprim/ sulfamethoxazole	116(48.9)	121(51.1)	200(76)	63(24)
Clindamycin	142(59.9)	95(40.1)	244(92.8)	19(7.2)
Amikacin	151(63.7)	86(36.3)	224(85.2)	39(14.8)
Chloramphenicol	84(35.4)	153(64.6)	210(79.8)	53(20.2)

DISCUSSION

In the present study, 500 isolates of *S. aureus* were collected. The site of isolation was maximum from pus and pus swabs (64.4%), followed by urine sample (14%) and blood (13%) and least from throat swabs (0.6%). Comparable results were seen in a study conducted by Vidya pai *et al*. [10], they reported prevalence of *S. aureus* in clinical specimens was maximum from pus (76.3%), least from body fluids (0.84%).

Table-3: Distribution of vancomycin mic values (e-test) among mrsa isolates

MIC value of vancomycin(µg/ml)	Number	Percentage (%)
2	1	0.42
1.5	46	19.4
1	39	16.4
0.75	57	24.1
0.5	52	21.9
0.25	42	17.7
Total	237	100

The rate of methicillin resistance was found to be 47.4% in *S. aureus* strains in this study. Furthermore, MRSA isolates were distributed among various clinical specimens as 56.5 % (maximum resistance) in pus and pus swabs followed by 36.9% in blood cultures, 31.4% in urine samples, 26.7% in sputum and least in high vaginal swabs (20%) and CSF (20%). Comparable results were reported by Kulshrestha *et al*. [11] in a study during 2013. They observed methicillin resistance in 51% of *S. aureus* strains. The maximum MRSA were isolated from pus (61%), followed by blood (15%), respiratory secretions (10%), Swabs and body fluids (5%) and least from urine (4%). In a study conducted by Joshi *et al*. [12] through INSAR group India, showed that overall prevalence of MRSA in India was 42% in 2008 and 40% in 2009. The rate of isolation of MRSA was maximum from pus followed by blood and respiratory specimens. This pattern is due to the reason that *Staphylococcus aureus* was isolated more in pus samples as it is one of the most common causes of the skin and soft tissue infections.

On the contrary, in the study by Bhatt *et al*. [13] in Nepal in 2011, they observed only 19% methicillin resistance. Maximum isolation of MRSA was from urine followed by blood and pus. In another study by Rajduraipandi *et al*. [14] in 2006, they observed methicillin resistance of 31.1% in clinical samples. The rate of isolation of MRSA was maximum from throat swab (35.7%) followed by pus (33.6%).

In the present study, all 237(100 %) MRSA isolates showed multidrug resistance. Multidrug resistance was defined as resistance of a MRSA strain towards three or more group of antibiotics except penicillin. Multidrug resistance in MRSA isolates has also been variable in different parts of the country. In the study conducted by Majumbder *et al*. [15] in 2001, they observed 23.2 % of MRSA isolates were MDR. Rajduraipandi *et al*.[14]. Observed that 63.6% of clinical MRSA isolates and 23.3% of carrier MRSA isolates showed multidrug resistance.Tiwari *et al*. [16] in 2008 in his study from Varanasi reported that 72.1% of MRSA isolates were MDR. In the study conducted by Sharma *et al*. [17] in 2011, reported 82.6% multidrug resistance. According to Indian literature, it seems that the burden of multi drug resistant-MRSA is increasing. The lesson is clear: MRSA surveillance and strict antibiotic policy are of paramount importance, which will lead to reduction in MRSA rate. In the present study, all the 237 MRSA isolates were screened for vancomycin resistance by vancomycin agar screen test and MIC values for vancomycin were determined by E test strips. However, none of the MRSA isolate grew on vancomycin agar screen and all the MRSA isolates had MIC of less than 2 µg/ml. All the MRSA isolates were vancomycin sensitive. 24.1 % MRSA isolates had Vancomycin MIC value of 0.75 and 21.9% of MRSA had Vancomycin MIC value of 0.5. Only17.7% isolates had MIC value of 0.25, 19.4% had MIC of 1.5, 16.4% had MIC value of 1 and only one isolate had MIC value of 2. So, VRSA/VISA is not prevalent in our hospital.

Comparable results were observed in the study conducted by Rengaraj *et al*. [18], there was no resistance observed to vancomycin by E test method. In their study, MIC of vancomycin ranged from 0.125µg/ml to 2µg/ml. According to Goyal *et al*. [19] in 2013, there was no vancomycin resistance observed by disc diffusion method.

On the contrary, in the study conducted by Tiwari *et al*. [20] in 2006 in Banaras, they observed that two *S. aureus* strains were found to be vancomycin resistant (one strain with MIC 32µg /ml and the other strain with MIC 64µg/ml);six strains of *S. aureus* were vancomycin intermediate (two strains with MIC 16 ml and four strains with MIC 8 µg/ml).Similarly, the study conducted by Bhattacharya *et al*. [21] in 2013 in kolkata, vancomycin resistance was seen in 21 isolates of *S. aureus* by disc diffusion method. Among them 4 isolates were found to be VISA when confirmed by E test with MIC varying between 4 to 6 mg/L. No VRSA was detected.

Though there are rare reports of VRSA and VISA in other parts of the country. It is suggested that the all MRSA isolates should be screened through MIC method for vancomycin resistance. This study highlights the need for continuous surveillance of antibiotic sensitivity pattern of *S. aureus* and MRSA. It would help in formulating and monitoring the antibiotic policy and ensures proper empirical treatment to patients. The use of standard and contact precautions by all healthcare workers in hospitals should be implemented to reduce the chance of MRSA infections

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