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PREVALENCE AND VARIATION IN REDUCED SUSCEPTIBILITY PATTERN OF NOSOCOMIAL MRSA ISOLATES TO VANCOMYCIN

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ABSTRACT

Staphylococcus aureus plays a vital role in hospital-acquired infection. It has overcome most of the antibiotics in the recent years. Their poly-antimicrobial resistance should be noticed carefully and dealt-with caution. Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a multidrug resistant group and are ubiquitous in the hospital environment. Vancomycin is a drug of choice for treating severe MRSA infections. However, the recent reports pointed about the reduction in the susceptibility to vancomycin observed among MRSA isolates. In our present study, an attempt was made to study the vancomycin susceptibility pattern of MRSA isolates from nosocomial infections during the period of October 2012–March 2013 and came out with fruitful results. Three isolates BS3, BS5 and SS2 showed intermediate sensitivity to vancomycin (VISA MIC 4–5µg/ml).The present study revealed the presence of MRSA with increasing vancomycin Minimum Inhibitory Concentration (MIC).This alarming result represents determination of MIC for vancomycin is necessary before starting the treatment for MRSA infections.

Keywords: MRSA; Vancomycin; MIC; Nosocomial infections.

INTRODUCTION

Nosocomial Infection (NI), also called "hospital-acquired infection", is defined as an infection which was not present or incubating when the patient is hospitalized and was acquired during the hospital stay [1].

Nosocomial Infection (NI) is an emerging problem in hospital practice. The rate of NI varies from 9.2% to 21.4% across different countries in the world. The rate of NI has been reported at 11.7% in Thailand, 17.0% in Ethiopia, 9.2% in UK and 9.0% in Norway. Prevalence of NI in post-operative patients was found to be higher (49.0%) than pre-operative patients (15.9%). Both Gram positive and Gram negative organisms play an important role in nosocomial infection. In bacterial analysis of hospital-acquired infection, Ashraf and Prodhan found that the predominant causative organisms for the post-operative wounds were *Escherichia coli* (37.5%), *Staphylococcus aureus* (21.7%), *Pseudomonas sp*. (15.1%), *Streptococcus sp*. (8.4%) and *Proteus sp*. (2.7%) in the surgery wards of Dhaka Medical College Hospital [2].

The genus *Staphylococcus* includes pathogenic organisms in which *S. aureus* is the most important pathogen those colonizes and infects both hospitalised patients with decreased immunity and healthy immuno-competent people in the community. The skin and mucous membrane are excellent barriers against local tissue invasion by *S. aureus*. However, if either of these is breached due to trauma or surgery, *S. aureus* can enter the underlying tissue, creating its characteristic local abscess lesion [3], and if it reaches the lymphatic channels or blood can cause septicaemia [4].

S. aureus is commonly associated with hospital- and community-acquired infections. It has overcome most of the therapeutic agents that have been developed against it in the recent years. The introduction of beta-lactamase resistant semi-synthetic Penicillins in the early 1960's provided temporary relief which ended with the emergence of Methicillin (Oxacillin)-resistant *S. aureus* (MRSA), discovered shortly after Methicillin became available for clinical use.

Infections caused by Methicillin or Oxacillin-resistant *S. aureus*are mainly nosocomial and are increasingly reported from worldwide. Vancomycin is the drug of choice for therapy of infections caused by MRSA, but increase in vancomycin use has led to the emergence of MRSA with reduced susceptibility to vancomycin. However in 2002, the first Vancomycin-resistant *Staphylococcus aureus* (VRSA) infection was documented in a patient in the United States [5]. There are two types of glycopeptide-resistant *Staphylococcus aureus*. The first one VISA (Vancomycin-intermediate *Staphylococcus aureus*) is due to thickened and poorly cross - linked cell wall. The second type,

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(Vancomycin - resistant *Staphylococcus aureus)* VRSA, is due to the acquisition from *Enterococcus sp.* of the *vanA* operon resulting in high-level resistance [6]. Due to increasing thrust in this area, we intended to perform the present study on "Prevalence and variation in reduced susceptibility pattern of nosocomial MRSA isolates to Vancomycin and the results may be helpful in treating MRSA.

MATERIALS AND METHODS

Sample Collection and Subjects Included in the Study

The patients with complaints of wound infection, fracture sites, respiratory and urinary tract infections admitted in wards and intensive care unit of Rajah Muthaiah Medical College and Hospital in Chidambaram, Tamilnadu, India were selected and included in this study. The patients suspected with hospital-acquired infections underwent clinical examination by qualified doctors. A total of 100 samples were collected from the patients suspected to have been infected. The samples were collected from fracture sites, surgical wound infection, respiratory tract infection, bacteremia and urinary tract infections.

Microbiological Methods

The specimens were inoculated onto blood and MacConkey agar plates and incubated at 37 C for 24–28 hrs. After enrichment, to screen *Staphylococcus sp*. from the samples, a loop full of inoculum was streaked onto Mannitol Salt Agar (MSA), a specific selective medium for *Staphylococcus sp.* and incubated. The cultures were identified by their characteristic appearance on their respective media, gram staining reaction and confirmed by the pattern of biochemical reactions using the standard method and the sensitivity of the detection increased by performing DNase test and Coagulase test [7].

Antibiotic Sensitivity Test

Methicillin-resistant *Staphylococcus aureus* isolates were screened using 1µgm of Oxacillin disc using Kirby Bauer method. All the isolates were tested for sensitivity against antimicrobial agents such as Vancomycin, Tetracycline, Chloramphenicol, Ampicillin, Erythromycin, Penicillin, Gentamycin and Rifampicin by disc diffusion method following CLSI guidelines and the resistance was confirmed on the basis of standard zone of inhibition [8].

Determination of MIC of Vancomycin

Minimum inhibitory concentration of vancomycin was detected by agar dilution method. The MIC values were recorded by observing the plates for lowest concentration of vancomycin that inhibited visible growth of the isolate. Muller–Hinton agar plates with increasing concentration of vancomycin ranging from 0.5 to 100µgm/ml were prepared and inoculated with the suspension of MRSA isolates.

RESULTS AND DISCUSSION

The present study was carried out from October 2012 to March 2013 in the Division of Microbiology, Faculty of Science, Annamalai University. The samples collected were of pus, blood, wound swab, sputum and urine from the patients suspected with nosocomial infections. Out of 100 isolates obtained, 52 isolates grew well in Mannitol Salt Agar and they were suspected as *Staphylococcus sp*.(Table1).

Nosocomial infections are considered as a serious threat in hospital practice. Mohiuddin *et al*. (2010) examined the microbiology of nosocomial infection in tertiary hospitals of Dhaka city. The predominant organisms responsible for nosocomial infections were *E. coli*(55.9%), *Pseudomonas* (33.3%), *Proteus*(12.7%), *Staph aureus* (5.9%).The isolated organisms showed high levels of resistance to commonly used antibiotics. All the 52 isolates were confirmed as S*taphylococcus aureus* based on phenotypic and biochemical characteristics including DNase and Coagulase tests. The percentage of total *Staphylococcus aureus* encountered from nosocomial cases was about 52% (Table 2).

Most of the microbes in the hospital environment have developed poly-antimicrobial resistance. The situation presented a challenge in maintaining good quality in patient case. We investigated antimicrobial susceptibility pattern of 52 isolates of *Staphylococcus aureus* against various antibiotics. Each isolate differed significantly in their susceptibility pattern for different antibiotics used. Interestingly, it was observed that all the 52 isolates were sensitive to vancomycin. Methicillin resistance of the isolates was screened and it was found that about 21 isolates exhibited resistance to Methicillin and were designated as MRSA and other isolates were designated as MSSA (Methicillin-Sensitive *Staphylococcus aureus*). The percentage of MRSA was 40%. MRSA isolates exhibited resistant against most of the antibiotics tested, whereas MSSA showed sensitivity to most of the antibiotics used in the present study. MRSA showed 100% resistance to Penicillin, 90% to Gentamycin, Rifampicin and Erythromycin. MSSA showed maximum resistance (50%) to Penicillin (Table 3).

Kaleem *et al*., evaluated *invitro* activities of different antibiotics against Methicillin-resistant *Staphylococcus aureus* (MRSA) [9]. Most of the MRSA strains were sensitive to Vancomycin, Tigecycline and Linezolid. Perwaiz *et al*., studied the antimicrobial susceptibility pattern of MRSA isolates from tertiary care hospital. They reported that 43% of the isolates were found to be MRSA and they were also resistant to many other antistaphylococcal antibiotics [10]. Rajaduraipandi *et al*., reported the prevalence and antibiotic susceptibility pattern of MRSA in major southern districts of Tamilnadu and confirmed that all strains of clinical and carrier strains were sensitive to Vancomycin. The determination of prevalence and antibiotic sensitivity pattern of MRSA will help the clinicians in first line treatment in referral hospitals [11]. Kamat *et al*., conducted a study among 498 patients from medicine and surgery wards in a tertiary teaching hospital in Goa. The patients were later checked for the occurrence of

nosocomial infections. The proportion of MRSA was 71.4%, 28.6% *Staphylococcus aureus* were sensitive to Methicillin [12].

Vancomycin has been the cornerstone of treatment for patients with serious MRSA infections for some decades and more than 99% of clinical *S. aureus* isolates remain susceptible to Vancomycin [13]. Some strains of MRSA with reduced susceptibility to Vancomycin were quoted by many authors. Sharma and Vishwanath found that out of 156 MRSA isolates tested,138 were susceptible to Vancomycin (VISA,MIC 0.5–2µgm/ml) and 18 isolates showed intermediate sensitivity to Vancomycin (VISA MIC 4–8µgm/ml) [14]. No Vancomycin-resistant *Staphylococcus aureus* (VRSA, MIC ≥ 16µgm/ml) was detected. In patients with *Staphylococcus aureus* bacteremia, higher Vancomycin MIC has been associated with prolonged bacteremia [15].

Table 1. Number of clinical samples collected from Nosocomial-infected patients

Sl. no	Source	Total no. of samples collected	No. of isolates grown in Mannitol Salt Agar
1	Pus sample	20	15
2	Blood	20	8
3	Sputum	20	9
4	Urine	20	10
5	Wound swab	20	10
	Total	100	52

Table 2. The percentage of MRSA from clinical samples

Sl. no	Source	Total *Staphylococcus aureus*	MRSA	Percentage
1	Pus sample	15	5	33
2	Blood	8	4	50
3	Sputum	9	3	33
4	Urine	10	4	40
5	Wound swab	10	5	50
	Total	52	21	40

Table 3. Susceptibility pattern of MRSA and MSSA against various antibiotics

Sl. No	Antibiotic	MRSA isolates (21)	MSSA isolates (79)
		No. of resistant isolates (%)	No. of resistant isolates (%)
1	Gentamycin	90	0
2	Rifampicin	90	2
3	Vancomycin	0	0
4	Ampicillin	81	6
5	Pencillin	100	50
6	Tetracyclin	85	5
7	Chloramphenicol	85	8
8	Oxacillin	100	0
9	Erythromycin	95	18

Table 4. Determination of MIC of Vancomycin against MRSA isolates

Sl. No	MRSA isolates	MIC values (µgm/ml)
1	BS3	4
2	BS5	5
3	SS2	5

Vancomycin-resistant *Staphylococcus aureus* (VRSA) are limited to a handful of reported cases, the rising MICs of Vancomycin among Vancomycin susceptible *Staphylococcus aureus* referred to as the Vancomycin MIC creep, has caused a re-evaluation of Vancomycin susceptibility criteria in cases of complicated infections like bacteremia or pneumonia [15-16]. In our present investigation, three isolates viz., BS3, BS5 (isolates from blood sample) and SS2 (isolate from sputum sample) showed intermediate sensitivity to Vancomycin (14%).These three VISA isolates recorded MIC values between 4 to 5µgm/ml (Table 4). Remaining 86% of the isolates were VSSA and their MIC values were between 0.5 to 2µgm/ml.

The results of the present study revealed that determination of antibiotic sensitivity through disc diffusion is not satisfactory for detecting VISA. The study also pointed out that there is a reduction in the susceptibility pattern of MRSA against Vancomycin is progressing or emerging slowly. So, as a precautionary measure it is of prime importance to determine MIC of such strains before starting treatment with Vancomycin, so that no VISA is missed and the emergence of Vancomycin-resistant strains could be minimized [17].

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