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Vancomycin resistance among methicillin resistant *Staphylococcus aureus* isolates from Doon Valley hospitals, Uttarakhand.

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ABSTRACT

Methicillin-resistant Staphylococcus aureus (MRSA) causing nosocomial infection posed a serious therapeutic challenge. We report the prevalence, antibiotic susceptibility pattern and MIC value of vancomycin of MRSA isolates from Doon valley hospitals, Uttarakhand. Clinical specimens were collected from different hospitals were subjected to MRSA screening using conventional microbiological methods. Subsequently the antibiotic sensitivity test was performed for the confirmed MRSA isolates and interpreted as per standard guidelines. The isolates were then checked for minimum inhibitory concentration (MIC) of vancomycin through broth macrodilution method. All MRSA strains were found to be multi-drug resistant including six isolates (15.7%) which showed resistance against vancomycin (VRSA) apart from resistant to other antibiotics. MIC detection of vancomycin further revealed six strains (15.8%) showed a high level of resistance (>32 µg /ml). Regular surveillance of hospital acquired infection excessive use of antimicrobial agents and monitoring of antibiotic susceptibility pattern is required to reduce the prevalence of MRSA.

Key words: MRSA, Uttarakhand , Vancomycin.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most important human pathogens responsible for a wide spectrum of infections, including skin and soft tissue infections, pneumonia, bacteraemia, surgical site infections (SSI), and catheter related infections [1] .MRSA first described in 1961 [2].Subsequently more and more incidents were reported worldwide [3].In recent years MRSA has emerged as one of the commonest cause of hospital acquired infection and continues to remain as an important factor [4]. Transmission of isolates of epidemic MRSA has traditionally been associated with hospital facilities [5]. A great deal of virulence from this organism occurs through cross-infection by spread from patient to patient in hospitals and other institutional settings. These Infected and colonized residents may also serve as potential sources for the spread of MRSA in long-term care facilities. Thus infected and colonized patients in hospitals mediate the dissemination of these isolates and hospital staff assists further transmission [6]. In contrast, healthy individuals have a small risk of contracting an invasive infection caused by *S. aureus*, but they can be carriers of the organism [7]. The prolonged hospital stay, indiscriminate use of antibiotics, lack of awareness, receipt of antibiotics before coming to the hospital etc. are the possible predisposing factors of MRSA emergence [8]. The infections caused by MRSA are difficult to treat. Only a few antimicrobial agents are available for treatment of such infections and none of these possesses ideal attributes. These infections has overcome most of the therapeutic agents that have been developed in the recent years and also

increased the use of glycopeptide vancomycin [9]. The development of resistance to multiple antibiotics and control of disease transmission by MRSA isolates in hospitals/communities have been recognized as the major challenges as the bacterial population that expresses the resistance phenotype varies according to the environmental conditions [10] .Therefore, the knowledge of prevalence of MRSA and their current antimicrobial profile become necessary in the selection of appropriate empirical treatment of these infections. In this study, we determined the prevalence, *in vitro* susceptibility pattern to various antimicrobial agents to record the current status of response to commonly used anti *Staphylococcus* antibiotics and MIC of vancomycin through macrobroth dilution method of isolated MRSA strains from different clinical screening samples in Doon valley hospitals.

MATERIALS AND METHODS

Sample processing and bacterial identification:

The study conducted on 300 inpatients of various hospitals of Doon Valley, North India during the period 2010-2011. *S. aureus* strains were isolated from nasal specimens and wounds collected from these patients after 48 h of admission to different wards such as general ward, burn ward, and dialysis ward. Specimens were inoculated onto mannitol salt agar (MSA), a selective medium for the isolation of *S. aureu* and incubated at 37^0C for 48 hours. Mannitol fermenting colonies (i.e. those that were yellow or gold) were selected from the MSA and sub-cultured on nutrient agar (NA) and colonies on NA were subjected to Gram's staining, catalase test and coagulase test. The Gram positive, catalase positive and coagulase positive isolates were considered as *S. aureus.* Oxacillin screen agar is used for the detection of methicillin resistance in *Staphylococci* as recommended by the NCCLS, 2000 [11]. The colonies were transferred to saline to produce a suspension that matches the turbidity of a 0.5 Mc Farland standard. This suspension was inoculated on the oxacillin agar screen plate Test plates which were incubated overnight at 35^0C and then examined.

Antibiotic susceptibility testing:

All the identified isolates of MRSA were undertaken in-vitro antibiotic susceptibility test by using Kirby-Bauer's disc diffusion method [12]. The bacterial suspension were prepared by inoculating the organism into 5ml peptone water and incubated at 37^0C for 16-20 hrs. The turbidity of the broth was adjusted equivalent to 0.5 McFarland standard (10^7 CFU/ml). 100ul of broth culture was then spread on the surface of Mueller - Hinton agar. The discs of different antibiotics (Hi-media) were placed on the surface of inoculated plates, which were then incubated for overnight at 37^0C. Plates were observed for zone of inhibition and measured in terms of inhibition zone diameter (IZD) in mm. The antibiotics used were ampicillin (10mcg/disc), ciprofloxacin (5mcg/disc), cefazolin (30mcg/disc), clindamycin (10mcg/disc), tetracycline (30mcg/disc), gentamycin (10mcg/disc), erythromycin (10mcg/disc), chloramphenicol (30mcg/disc) , linezolid (30mcg/disc) and vancomycin (30mcg/disc). *S. aureus* ATCC 25923 was used as a quality control strain.

Determination of minimum inhibitory concentration (MIC):

In view of the fact that VISA are detected only by dilution-based susceptibility test methods [13] MIC of vancomycin for all the 38 strains was determined by tube dilution method (macrobroth dilution) [14] – twofold dilutions of vancomycin were prepared in Mueller-Hinton broth (HiMedia, Mumbai) ranging from 0.25-128 µg /mL. To each tube 1mL of actively growing suspension of MRSA was added, the inoculum was prepared adjusting the concentration of MRSA to 10^5 - 10^6 colony forming units (CFU)/mL using McFarland standard and the results recorded were after 24 hours of incubation at 35°C.

RESULTS

Of the 300 clinical samples studied, *S. aureus* was isolated from 111 samples (37%). Out of these 111 *S. aureus* isolates, 38 (34.2%) were methicillin resistant (MRSA) through Oxacillin screen agar test. Demographically analysis of data shows 25 (38%) and 13 (29%) of MRSA isolates were from male and female respectively. MRSA isolates showed a high level of resistance to all antimicrobials in general. All strain were resistant to ampicillin and cefazolin. High resistance was noted for gentamycin (92%), tetracycline (81.5%), erythromycin (73.7%) and ciprofloxacin (61.5%). The isolates showed moderate resistance to chloramphenicol (44.7%) and linezolid (31.6%) and low rates of resistance to vancomycin (15.7%). Detailed description is given in table-I. A high level of resistance (>32 µg /ml) was noted in 6 (15.8%) isolates and low level resistance (<4 µg /ml) in 1 (2.6%) by tube dilution method for MIC of vancomycin indicated in Table-II. Thus six strains are vancomycin resistant

Staphylococcus aureus (VRSA) which is also shown by disc diffusion method. Some VRSA strains were sensitive to linezoid and other cases to chloramphenicol as shown in table III.

Table I. Antibiotic susceptibility pattern of MRSA isolates (n=38).

Antibiotic	Disc content	No. of resistant isolates	% Res
Ampicillin	10mcg/disc	38/38	100
Ciprofloxacin	5mcg/disc	23/38	60.5
Cefazolin	30mcg/disc	38/38	100
Tetracycline	30mcg/disc	31/38	81.5
Gentamycin	10mcg/disc	35/38	92
Erythromycin	10mcg/disc	28/38	73.7
Vancomycin	30mcg/disc	06/38	15.7
Linezolid	30 mcg /disc	12/38	31.6
Chloramphenicol	30 mcg /disc	17/38	44.7
Clindamycin	10 mcg /disc	20/38	52.6

Table II. Distribution of vancomycin MIC values among MRSA isolates (n=38)

MIC value of vancomycin (µg/mL)	Number	Percent (%)
128	2	5.2
64	3	7.9
32	1	2.6
16	0	0.0
8	0	0.0
4	1	2.6
2	5	13.1
1	9	23.6
0.5	6	15.7
0.25	11	28.9
Total	38	100

Table III. Resistance pattern of vancomycin resistant staphylococcal isolates to commonly tested antimicrobial agents as determined by disc diffusion method

Strain no.	Antibiotic									MIC for VAN (µg /mL)	
	AMP	CEF	CIP	TET	GEN	ERY	CHL	LEN	CLI	VAN	
22	R	R	R	S	R	R	R	S	R	R	128
56	R	R	R	R	R	R	R	S	R	R	128
77	R	R	S	R	R	R	R	S	R	R	64
114	R	R	S	R	R	R	R	S	R	R	64
208	R	R	S	R	R	R	R	S	R	R	64
267	R	R	S	R	R	R	S	S	R	R	32

Abbreviations: AMP, ampicillin;; CHL, chloramphenicol; CIP, ciprofloxacin; CLI, clindamycin; ERY, erythromycin; GEN, gentamicin; VAN, vancomycin; LEN Linezoid; CEF, cefazolin; TET, tetracycline; R, resistant; S, sensitive.

DISCUSSION

MRSA is a major nosocomial pathogen causing significant morbidity and mortality [15]. *S. aureus* is one such pathogen that has shown tendency to develop resistance to a number of antimicrobials and MRSA is one such group that has been a matter of concern since late 1970s. Susceptibility test profile revealed a high level of resistance amongst the *S. aureus* to most of the commonly used antimicrobials. The development of antibiotic resistance in developing countries like ours seems to be very much related to the irrational antibiotic usage due to its easy availability at the drug store without prescription, injudicious use in hospitals and uncontrolled use in agriculture, animal husbandry and fisheries .

In the present study, all strains were resistant to ampicillin and cefazolin. The high level of resistance to ampicillin, tetracycline, erythromycin and ciprofloxacin can be attributed to the fact these antibiotics are frequently used in the treatment of common infections. A higher rate of clindamycin resistance in endemic isolates is disappointing as this drug has a very good efficacy in MRSA SSTI [16]. This may be due to the increased use of antibiotics.

Some strains also showed resistance against vancomycin which is an alarming state. Vancomycin is currently the main antimicrobial agent available to treat infections with these MRSA. But widespread use of vancomycin to treat

infections caused by MRSA and other gram-positive cocci had lead to the emergence of vancomycin resistance *S. aureus* (VRSA). In the present study, VRSA showed resistance to a wide range of antimicrobial agents which include ampicillin, cefazolin, ciprofloxacin, clindamycin, erythromycin, gentamycin, tetracycline and vancomycin. However, it was susceptible to linezolid and chloramphenicol as determined by Kirby-Bauer's disc diffusion method (Table 2). Linezolid and quinupristin/dalfopristin were recently approved by the Food and Drug Administration and are antimicrobials with activity against glycopeptides-resistant Gram positive microorganisms such as VRSA [17]. Resistance to chloramphenicol was average (44%). There are certain trials of usage of chloramphenicol in multi-drug resistant gram positive organisms like MRSA, vancomycin-intermediate *Staphylococcus aureus* (VISA), vancomycin-resistant *Staphylococcus aureus* (VRSA) and vancomycin-resistant enterococcus (VRE) [18] Thus,the emergence of the glycopeptide resistance is of great concern. Though first case of VRSA was reported in 2002 in USA [19] Recently Bathaineh has reported VRSA strains from Jordan [20]. Ashdulla et al have found some strains of vancomycin intermediate *S. aureus* (VISA) from India. Song et al, 2004 have also been reported the emergence of vancomycin resistant *S. aureus* strains from India and its neighboring countries [21].Tiwari and sen (2006) also reported isolation of VRSA strain from northern India. Similarly Venubabu Thati etal (2011) isolated VRSA strain from Hydrabad south India.

In our study, we found six (15.7 %) strains exhibiting MIC of vancomycin higher than 32µg/mL. This may be a pointer towards emerging high level vancomycin resistance in *S. aureus*. Though chloramphenicol and linezolid can be effective if used rationally. Also patients colonized but not infected with MRSA should not be treated with vancomycin merely for the sake of eliminating carriage of this organism. Similar studies should be carried out by more and more hospitals so that appropriate measures are taken whenever and wherever necessary.

CONCLUSION

Detection of vancomycin resistance in *S. aureus* isolates emphasizes strict regulation on irrational antibiotic usages. The increase of vancomycin resistance among MRSA and excessive use of antimicrobial agents leads to high-level glycopeptide resistance in clinical settings which proclaim further epidemiological studies. When antimicrobials including vancomycin are considered for treatment, there is need for *in vitro* susceptibility testing of every isolate of MRSA in the clinical laboratories and an effective infection control policy to prevent the spread of VRSA in the healthcare facilities.

REFERENCES

[1] N De San, O Denis, MF Gasasira, R De Mendonça, C Nonhoff, MJ Struelens. *J Clin Microbiol* **2007**;45(4):1098-1001.
[2] MP Jevons. *Br Med J* **1964**;1:124-6
[3] PJ Sanderson. *Br Med J* **1986**; 293:573-4.
[4] 4 S Salmenlinna, O Lyytikainen, J Vuopio-Varkila. *Emerging Infect Dis* **2002**; 8: 602-7.
[5] RL Thompson, I Cabezudo, RP Wenzel. *Ann Intern Med* **1982**; 97: 309-17.
[6] AH Qureshi, S Rafi, SM Qureshi, AM Ali. *Pak J Med Sci* **2004**;20:361–4.
[7] TJ Foster. *J Clin Invest* **2004**; 114: 1693-6.
[8] S Anupurba, MR Sen, G Nath, BM Sharma, AK Gulati, TM Mohapatra. *Indian J Med Microbiol* **2003**;21:49–51.
[9] GA Oliveira, AM Dell'Aquila, RL Masiero, CE Levy, MS Gomes, L Cui, et al. *Infect Control Hosp Epidemiol* **2001**; 22(7):443-8.
[10] McDonald M. *Aust N Z J Surg* **1997**;67**:**682–5.
[11] NCCLS. Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria. 5th Edn., **2000**,Villanova, Pa.
[12] JH Ortez. Disk Diffusion Testing. In Coyle MB, coordinating editor. Manual of Antimicrobial Susceptibility Testing. *American Society for Microbiology* **2005**: 39-52.
[13] L Verbist. *Eur J Microbiol Infect Dis* **1993**;12(Suppl .1):2-5.
[14] Ericsson. Antibiotic. *Acta Pathol Microbiol Scand Soet B Supple* **1971**;l217:3-9.
[15] D Sachdev, S Amladi, G Nataraj, S Baveja, V Kharkar, S Maharajan, *et al*. *Indian J Dermatol Venereol Leprol* **2003**;69**:**377– 80.
[16] CJ Baker, RW Frenck. *AAP News* **2004**; 25; 105.
[17] ES Schweiger, NS Scheinfeld, HR Tischler, JM Weinberg. *J Drugs Dermatol* **2003**; 2 : 378-83.

[18] M Niks, J Hanzen, D Ohlasová, D Rovná, A Purgelová, Z Szövényiová, et al. *Klin Mikrobiol Infekc Lek* **2004**; 10:124-29.
[19] Centers for Disease Control and Prevention. *Morb Mortal Wkly Rep MMWR* **2002**, 51:565-567.
[20] AB Bataineh. *Pak J Med Sci* **2006**, 22:144-148
[21] JH Song, K Hiramatsu, JY Suh, KS Ko, T Ito, M Kapi et al. *Antimicrob Agents Chemother* **2004**, 48:4926-4928.