

Vancomycin Resistance among Methicillin Resistant *Staphylococci* Isolated from Different Clinical Samples at Tertiary Care Hospital

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Received on 29.01.2019, **Accepted on** 07.03.2019

Abstract

Background: The glycopeptide vancomycin was considered to be the best alternative for the treatment of multi drug resistant MRSA. However, there are increasing numbers of reports indicating the emergence of vancomycin-resistant *Staphylococcus aureus* (VRSA) strains exhibiting two different resistance mechanisms. The emergence and spread of resistance to vancomycin is a threat to the already challenging therapy of MRSA. **Materials and Methods:** The present study was carried out to find out the presence of VISA and VRSA in the tertiary hospital. A total 570 *staphylococcus aureus* isolates consisting of 340 MRSA and 230 MSSA were isolated from different clinical specimens from various outpatient departments and wards using the standard techniques. All MRSA isolates were subjected to disc diffusion testing and MIC testing against vancomycin. **Result and Discussion:** Out of the 340 MRSA isolates, 270 isolates were Vancomycin sensitive *Staphylococcus aureus* (VSSA) (MIC: 0.5-2µg/ml), 70 isolates were Vancomycin intermediate *Staphylococcus aureus* (VISA) (MIC: 4-8µg/ml) and none were Vancomycin resistant *Staphylococcus aureus* (VRSA) (MIC: >16µg/l). The present study reveals for the first-time emergence of VISA/VRSA from this part of Karnataka, India.

Keywords: *Staphylococcus Aureus*; MRSA; Vancomycin; VRSA; VISA.

How to cite this article:

Siddesh KC, Ashrith KC, Halesh L H. Vancomycin Resistance among Methicillin Resistant *Staphylococci* Isolated from Different Clinical Samples at Tertiary Care Hospital. J Microbiol Relat Res. 2019;5(1):45-49.

Introduction

The emergence of high levels of penicillin resistance followed by the development and spread of strain resistant to the semisynthetic penicillins (methicillin, oxacillin, and nafcillin), macrolides, tetracycline, and aminoglycosides has made the therapy of *staphylococcal* disease a global challenge [1]. *Staphylococcus aureus*, a major cause of potentially life-threatening infections acquired in health care and community settings, has developed

resistance to most classes of antimicrobial agents. A dramatic increase in the number of health care-associated infections due to methicillin-resistant *Staphylococcus aureus* (MRSA) in the 1990s and the recent emergence of MRSA in community-associated infections highlight the success of this species as a pathogen and its ability to adapt under pressure from antimicrobial agents [2].

Methicillin resistant *S.aureus* (MRSA) was first detected in 1961 and has occurred in many countries. Even after 40 years it is still among the top three

clinically important pathogens [3,4]. However, in recent years, clinicians have been concerned by the increased frequency of MRSA infections.

The glycopeptide vancomycin was considered to be the best alternative for the treatment of multi drug resistant MRSA [5,6]. However, there are increasing numbers of reports indicating the emergence of vancomycin-resistant *Staphylococcus aureus* (VRSA) strains exhibiting two different resistance mechanisms.

Initially vancomycin intermediate *Staphylococcus aureus* (VISA) noted in Japan in 1996 and subsequently in United States in 1997 was believed to be due to the thickened cell wall [7,8]. The second, noted in United States in 2002 [9] among *Staphylococcus aureus*, was identical to the mechanism seen in vancomycin-resistant enterococcus [10]. Vancomycin resistant *Enterococcus faecium* harbors the vanA operon, which contains five genes, VanS, -R, -H, -A and -X [10]. But Tiwari and Sen have reported a VRSA which is van gene-negative [11]. Subsequent isolation of VISA and VRSA isolates from other countries including Brazil [12], France [13], United Kingdom [14], Germany [15], India [11,16] and Belgium [17] has confirmed that the emergence of these strains is a global issue.

Materials and Methods

The present study was a cross sectional study carried out in the Department of Microbiology, Shimoga Institute of Medical Sciences, Shimoga, Karnataka, during August 2014 to July 2017. Various clinical samples like pus, blood and urine from the patients attending outpatient Departments and from those admitted to wards of Surgery, Medicine, Orthopedics and Obstetrics and Gynecology in McGann Teaching Hospital, Shimoga were used for the study. Specimen were collected in a sterile, proper labelled container with aseptic precautions and processed as per standard Microbiological procedures.

Staphylococcus aureus isolates were identified by their colony morphology, Gram stain and various biochemical tests by standard conventional techniques. Methicillin resistant *Staphylococcus aureus* (MRSA) strains were identified by phenotypic methods by Kirby Bauer disc diffusion method and interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines.

Disc diffusion method

Vancomycin resistance was detected by disc diffusion method using Vancomycin discs (30 µg) as per CLSI guidelines and interpreted as per the manufacturer guidelines (Hi-Media Laboratories, Mumbai)

Determination of MIC by agar diffusion method using HiComb strips (Hi-Media Laboratories private limited, Mumbai):

- This strip is intended only for agar diffusion method and not for broth dilution method
- Plates were prepared with Mueller-Hinton agar for rapidly growing aerobic organism as per Kirby-Bauer method. Only pure cultures were inoculated.
- A sterile non-toxic cotton swab on a wooden applicator was dipped into the standardized inoculum and the soaked swab was firmly rotated against the upper inside wall of the tube to express excess fluid.
- The entire agar surface of the plate was streaked with the swab three times, turning the plate at 60° angle between each streaking.
- The inoculum was allowed to dry for 5-15 minutes with lid in place.
- The HiComb MIC strip was applied to the agar surface with MIC scale facing upwards as per manufacturer's instructions. Once applied the strip was not moved and was let to absorb to the surface of agar media.
- The agar plate was incubated at 35-37°C and examined after 18-24 hours.

Interpretation

The zone of inhibition was in the form of an ELLIPSE. MIC value would be the value at which the zone convenes the comb like projections of the strips and not at the handle.

- If there were no zone of inhibition observed, the MIC was reported as greater than the highest concentration on the strip.
- If zone of inhibition was below the lowest concentration then the MIC was reported as less than the lowest concentration. Readings were taken according to manufacturer's instructions as MIC of $\leq 2\mu\text{g/ml}$ - Susceptible (VSSA), MIC of $4-8\mu\text{g/ml}$ - Intermediate (VISA) and MIC of $\geq 16\mu\text{g/ml}$ - Resistant (VRSA).

Results

In the present study, a total of 550 clinical samples were collected from various clinical cases. Out of 550 cases, 297 (54%) patients were male and 253(46%) were female patients. The sex distribution is shown in Table 1.

Table 1: Gender wise distribution of clinical samples used in the study

Gender	Number	Percent (%)
Male	297	54
Female	253	46
Total	550	100

Out of the total 550 various clinical samples, namely from pus/exudates (396), blood (33) and urine (121), 313 were *Staphylococcus aureus*. Majority of the *S. aureus* isolates were from pus followed by blood and then urine.

Of the 313 *Staphylococcus aureus* isolates, 187 (59.75%) were Methicillin Resistant *Staphylococcus aureus* and 126 (40.25 %) were Methicillin Sensitive *Staphylococcus aureus*. Of the 187 MRSA isolates, 183 were found in pus and 4 were found in blood whereas none were found in urine.

Table 2: Details of various clinical samples from which the *staphylococcus aureus* was isolated.

Clinical Specimen	No. of samples taken	<i>Staphylococcus aureus</i>	
		Number	Percentage
Pus	396	298	95
Blood	33	09	3
Urine	121	06	2
Total	550	313	100

In the present study, out of 187 Methicillin Resistant *Staphylococcus aureus* (MRSA), all the isolates were susceptible vancomycin by disc diffusion method with varying zone of inhibition. Minimum inhibitory concentration was calculated by HiComb MIC test method (HiMedia Laboratories Pvt. Limited). Out of the 187 MRSA isolates, 148 isolates were Vancomycin sensitive *Staphylococcus aureus* (VSSA), 39 isolates were Vancomycin intermediate *Staphylococcus aureus* (VISA) and none were Vancomycin resistant *Staphylococcus aureus* (VRSA). All 39 VISA isolates were isolated from pus and exudates.

Antibiotic susceptibility pattern showed that all MRSA strains were resistant to oxacillin and cefoxitin. Also, they were resistant to most of the antibiotics tested and all MRSA strains were susceptible to vancomycin

Table 3: Specimen wise distribution of MRSA

Clinical specimen	MRSA	
	Number	Percentage
Pus / Exudates	183	97.86
Blood	04	2.14
Urine	0	0
Total	187	100

Discussion

In the present study 550 clinical samples were collected. Out of which 396 samples were pus, 121 urine samples and 33 were blood samples. Of the 313 *Staphylococcus aureus* isolated, 187 (59.75%) were found to be methicillin resistant.

The reported percentage of MRSA isolation from clinical specimens by different workers varies over a wide range as shown in the table 4.

In a study conducted by Thati et al in Hyderabad, out of 358 clinical isolates of *Staphylococcus aureus*, 285 (79.6%) were identified as Methicillin resistant *Staphylococcus aureus* (MRSA) by disc diffusion method. Our study has also shown MRSA incidence of more than 50%. In another study conducted by Indian Network for Surveillance of Antimicrobial Resistance (INSAR), the percentage of MRSA isolated among *Staphylococcus aureus* isolates was 41%. Tiwari et al. (2006), Institute of Medical Sciences, Banaras Hindu University, Varanasi found out the presence of VISA and VRSA in the northern part of India, the percentage of MRSA isolated for a period of three years from August 2002 to July 2005 was 40.61%. Present study has shown highest numbers of MRSA (183) are isolated from pus followed by blood. Chakravarthy A et al [19], Mehta A.P. et al [20] and Pal N. and Ayyagiri A. [21] have also reported maximum number of MRSA isolation from pus. All the MRSA isolates were resistant to penicillin and Ampicillin. MRSA isolates were significantly more resistant (in numbers) to all the tested antibiotics except vancomycin and linezolid. The glycopeptide vancomycin was considered to be the best alternative for the treatment of multi drug resistant MRSA. However, there are increasing numbers of reports indicating the emergence of vancomycin-resistant *S. aureus* (VRSA) strains.

In the present study, vancomycin susceptibility was detected by Kirby-Bauer's disc diffusion method and MIC was determined by HiComb MIC test (HiMedia Laboratories, Mumbai, India). All strains were susceptible to vancomycin by disc diffusion method.

Table 4: Percentage of MRSA samples used by various authors in the previous reports.

Sl. No.	Author	Place	Year	% MRSA
1.	Furuno et al. [22]	Baltimore, USA	2003	9.83
2.	Tiwari et al. [11]	Varanasi, India	2006	40.61
3.	Indian Network for Surveillance of Antimicrobial Resistance (INSAR) [21]	India	2009	41.00
4.	Thati et al. [10]	Hyderabad, India	2011	79.60
5.	Present Study	Shimoga, India	2017	59.65

Table5: Percentage of VSSA samples used by various authors in the previous reports.

Sl.No.	Author	Place	Year	VSSA (%)
1.	Assadullah et al. [16]	Srinagar, India	2003	81.70
2.	Reuf C. [26]	USA	2004	77.00
3.	Tiwari et al. [11]	Varanasi, India	2006	97.48
4.	Thati et al. [10]	Hyderabad, India	2009	93.57
5.	Present Study	Shimoga, India	2017	79.14

Table 6: Percentage of VISA samples used by various authors in the previous reports.

Sl.No.	Author	Place	Year	%VISA
1.	Assadullah et al. [16]	Srinagar, India	2003	15.00
2.	Reuf C. [26]	USA	2004	20.00
3.	Tiwari et al. [11]	Varanasi, India	2006	1.89
4.	Thati et al. [10]	Hyderabad, India	2009	4.47
5.	Present Study	Shimoga, India	2017	20.85

Widespread use of vancomycin to treat infections caused by MRSA has been reported to result in the emergence of low-level resistance. Vancomycin was used clinically in treating infections in our hospital during study period. This could be the reason for detecting vancomycin resistance in MRSA isolates in the present study.

Previous studies done by Assadullah et al, Tiwari et al, Thati et al have reported 81.7%, 97.48% and 93.5% susceptibility to Vancomycin. 79.41% susceptibility to Vancomycin was found in the present study (Table 5). In the present study, 39isolates (20.85%) showed MIC of 4-8 µg/ml which were identified as Vancomycin Intermediate *Staphylococcus aureus* (VISA, Table 6).

Conclusion

Despite the recent reports that gram negative bacterium has overtaken *staphylococci* because the leading explanation for health facility infections, MRSA continues to be the main threat in the health care setting.

Prior publication: No

Support: Nil

Conflicts of interest: None

Permissions: Not applicable

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