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REGIONAL OCCURRENCE, ANTIBIOGRAM- RESISTOGRAM AND VIRULENCE PATTERNS OF CARRIER - DERIVED VANCOMYCIN RESISTANT *STAPHYLOCOCCUS AUREUS* (VRSA)

Beerbal Kumar B., Panneer Selvam K.*, Nambirajan G., Rajan S. and and Reshma A.

PG & Research Department of Microbiology, M. R. Government Arts College, Mannargudi - 614 001.

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***Corresponding Author**
Panneer Selvam K.
PG & Research Department of Microbiology, M. R. Government Arts College, Mannargudi - 614 001.

ABSTRACT

Vancomycin resistant *Staphylococcus aureus* (VRSA) are the most important drug resistant pathogens of public health concern and are currently reported frequently compared to earlier times. Though vancomycin has been considered as the drug of choice for the treatment of methicillin resistant *S. aureus* infections, the therapy became a challenge after the emergence of vancomycin staphylococcal isolates. Against the fact that *S. aureus* cause serious diseases from surgical wound infections to blood stream infections, their reported carriage by healthy persons, emerging resistance to a significant number of antibiotics including vancomycin, the treatment of such infections has become more difficult. As regular screening of healthy persons for *S. aureus* with special reference to VRSA carriage and determination their antibiotypes would benefit the health care providers to prevent/ control and treat infections by VRSA promptly, the screening carried out during the present study brought out the fact that as much as 15 (5.5%) of 272 carrier- derived *S. aureus* were identified to be resistant to vancomycin possessing a diverse antibiotic susceptibility patterns/ virulence features.

KEYWORDS: *Carrier- derived VRSA, susceptibility testing, antibiotypes, virulence.*

INTRODUCTION

Vancomycin is an effective drug of choice to treat infections caused due to *Staphylococcus aureus* strains that are resistant to methicillin and in this context, reports of vancomycin resistant *S. aureus* (VRSA) across the globe is a serious clinical/ health concern. More

importantly, the antibiotic was the most reliable therapeutic compound against methicillin-resistant *S. aureus* (MRSA) caused human infections until 1996, as MRSA isolate resistant to vancomycin was reported from a patient in Japan during the year. Though a majority of *S. aureus* isolates are susceptible to vancomycin and against this back ground the drug was in use to treat many serious bacterial infections, an injudicious/ excessive use (CDC, 2002) of vancomycin to treat MRSA infections has been the root cause of emerging VRSA pathogens and poses a threat to the human health care. The treatment of infected cases is noted to be clinically difficult as the illness could range from skin infections to severe invasive diseases like pneumonia/ septicemia, though infection by VRSA is infrequent. In 1998, Lessing *et al*. reported a VRSA strain isolated from a 32- years-old male with hepatic cirrhosis patient displaying a vancomycin minimum inhibitory concentration (MIC) as high as >32µg /ml and the patient was reported to be treated with a combination of trimethoprim/sulfamethoxzole. This is more significant against the report of Sakoulas *et al.* (2004) that for isolates with a vancomycin MIC >2µg/ml, an alternative/ substitute to vancomycin was used based on *in vitro* susceptibility testing. A 2002 (Brandi et al.) report brought out a first case of a fully vancomycin resistant isolate of MRSA from USA. It also was observed that the most susceptible were the persons with chronic health conditions and previous MRSA infections, higher was their vulnerability to VRSA infections. Similarly, Loomba et al. (2010) reported that a close physical contact with infected patients/ contaminated materials could promote VRSA infections. Against this clinical significance, the present study was carried out at the PG & Research Department of Microbiology, M. R. Government Arts College, Mannargudi, to bring out the regional occurrence/ prevalence of carriage rate of *S. aureus* with special reference to carrier- derived VRSA as well as their susceptibilities to a panel of antibiotics.

MATERIALS AND METHODS

The study carried out at the PG & Research Department of Microbiology, M.R. Government Arts College, Mannargudi, Tamilnadu state, India, initially undertook a survey so as to bring out the general health awareness practiced among the adolescent learners of higher education. Further, nasal and fingertip swabs were collected aseptically for bacteriological evaluations from volunteers as recommended by Oslen *et al*. (2012) and were processed based on standard microbiological processes.

The basic epidemiological (on staphylococcal carriage) information such as age, sex, location, current/ previous treatment taken etc., were consolidated based on the

recommendations of Brown *et al.* (2005) & Collee *et al.* (1996) and were suitably evaluated. During the study, the isolates of *S. aureus* were identified based on Gram's reaction, culture/ specific growth characteristics/ biochemical characteristics. Precisely, all the preliminarily identified isolates of *S. aureus* were further confirmed by coagulase test as well as by other recommended biochemical tests along with a control strain of *S. aureus* MTCC 3160. The procedures outlined by the Clinical Laboratory Standards Institute (CLSI, 2007) were strictly followed for the identification and confirmation of *S. aureus*.

Antibiotic susceptibility patterns of VRSA (CLSI, 2014)

The Kirby- Bauer disc diffusion method derived susceptibilities of all the vancomycin resistant *S. aureus* of the study were consolidated/ evaluated. The susceptibilities against fourteen various antibiotics [Penicillin (10 µg), Oxacillin (1µg), Cefoxitin (30 µg), Vancomycin (10 µg), Gentamycin (10 µg), Tobramycin (10 µg), Tetracyclin (30 µg), Ciprifloxacin (5 µg), Levofloxacin (5 µg), Ofloxacin (5 µg), Moxifloxacin (5 µg), Norfloxaxin (10µg), Gatifloxacin (5 µg) and Co- Trimoxazole (25µg)] belonging to ten groups at varying concentrations were analyzed. In addition, the test carrier- derived VRSA isolates were ascertained for their susceptibility based on vancomycin agar screening methods. Further, the isolates of VRSA were also tested for catalase and coagulase (slide and tube methods) activities, sugar fermentation, pigment production onto nutrient agar, hemolytic activities on sheep, coat, chicken and human blood agar, biofilm formation and gelatinase activity and were analyzed. Similarly, the virulence factors such as DNase activity, thermonuclease and phosphotase activity were also assessed among the test VRSA isolates.

RESULTS AND DISCUSSION

A total number of 2480 anterior nares (n= 1240), palm (n= 620), index finger (n= 566) and dorsum (n= 54) derived swab samples collected from respectively 116 and 504 male and female volunteering learners were processed based on standard microbiological process. Further, it was also observed that mostly female learners were reported with frequent illnesses compared to male volunteers and a few even reported either recent or current use of antibiotics. In particular, of the total volunteers, as much as 3 and 12 male and female persons respectively were found with VRSA carriage and a total of 15 vancomycin resistant *S. aurues* (VRSA) were confirmed in the study. Specifically, among the 15 VRSA isolates 26.6% (n= 4) and 20% (n= 3) were reported respectively with some health disorder and with antibiotic

therapy. While the study identified 5.5% of 272 *S. aureus* isolates as vancomycin resistant, Sarrafzadeh et al. (2001) reported 9.2% of their isolates as VRSA. Further, the number of palm derived VRSA was noted to be higher (33.3% 5 of 15 isolates) compared to the VRSA from other regions [direct finger (4; 26.6%), right (2; 13.3%) and left (4; 26.6%) nares] of the volunteers. It was also noted that as much as 7 of the 15 VRSA isolates were identified from persons who represented a particular taluk – Mannargudi and the rest of the isolates were confirmed from volunteers representing other regions of the study.

The antimicrobial susceptibility patterns (table 1) as well as the virulence features among the 15 VRSA isolates were observed to be diverse. Alarmingly, 8 (53.3% of 15) VRSA isolates were noted to be MRSA as the isolates displayed no susceptibility either with oxacillin and cefoxitin discs or with oxacillin agar screening method. In particular, all the isolates were susceptible to gentamycin and tobramycin. While penicillin inhibited an isolate of VRSA, 3 (20% of 15) isolates of VRSA were inhibited by gatifloxacin. Similarly, all the isolates were susceptible to tetracycline and Ciprofloxacin, Levofloxacin, Ofloxacin and Norfloxaxin were inhibitive against 93% of VRSA isolates of the study.

Further, 80% (12 of 15) of the isolates found to form biofilm as was noted on congo red agar and as much as 67% was positive for coagulase production in tube method. In addition, though 33.3% (n=5), 66.6% (n= 10) and 53.3% (n=8) of the isolates had possessed DNase, Phosphatase and Gelatinase activities respectively, none of the isolates displayed thermonuclease activity. Also, 6 & 1 of 15 VRSA had respectively beta and alpha haemolysin activities on sheep blood agar. According to Bobin *et al.* (2001), vancomycin resistance was not necessarily confined to MRSA and that the mechanism of resistance in vancomycin resistant/ intermediate *S. aureus* strains could involve exclusive alterations in cell wall metabolism, which thus could affect the activities of β- lactam antibiotics.

Table 1: Carrier- derived vancomycin resistant S. aureus (VRSA; n= 15) and their antibiotypes.

No.	*Antibiotics*	*Total no. of VRSA and % of antibiotypes*					
		Susceptible		*Intermediate resistance*		*Resistance*	
		No.	*%*	*No.*	*%*	*No.*	*%*
1	Penicillin (P) (10 µg)	1	(6.6%)	-	-	14	(93.3%)
2	Oxacillin (OX) (1µg)	14	(93.3%)			1	(6.6%)
3	Cefoxitin (CXX) (30 µg)	11	(73.3%)	2	(13.3%)	2	(13.3%)
4	Vancomycin (VA) (10 µg)	0	0			15	100%
5	Gentamycin (GEN) (10 µg)	15	100%	-	-	0	-
6	Tobramycin (TET) (10 µg)	15	100%	-	-	0	-
7	Tetracyclin (TET) (30 µg)	12	(80 %)	2		1	(6.6%)
8	Ciprofloxacin (CIP) (5 µg)	14	(93.3%)	-	-	1	(6.6%)
9	Levofloxacin (LEV) (5 µg)	14	(93.3%)	-	-	1	(6.6%)
10	Ofloxacin (OF) (5 µg)	14	(93.3%)	-	-	1	(6.6%)
11	Moxifloxacin (MO) (5 µg)	13	(86%)	1	(6.6 %)	1	(6.6%)
12	Norfloxaxin (NX) (10µg)	14	(93.3%)	1	(6.6 %)	-	-
13	Gatifloxacin (GAT) (5 µg)	3	(20%)	2	(13.3%)	10	(66.6%)
14	Co- Trimoxazole (COT) (25µg)	14	(93.3%)	0	-	1	(6.6 %)

CONCLUSION

Against the fact that fifteen of the 272 carrier- derived *S. aureus* were actually resistant to vancomycin, the present study is alarming in terms of both prevention of VRSA dissemination as well as treatment of infected cases. Additionally, the existing antibiotics seemed to be unable in fighting such bacterial strains, more meaningful and effective precautionary measures are the dire needs of community and nosocomial associated bacterial infections. A higher awareness amongst the public & healthcare providers on carrier status, public response/ commitment, appropriate follow-up by clinical settings/ researchers are to be given priority towards prevention/ management of transmission as well as therapy of virulent pathogens such as vancomycin resistant *S. aureus*.

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