ORIGINAL ARTICLE

Determination of Vancomycin, Teicoplanin and Linezolid resistance among Staphylococcal isolates from a tertiary care hospital

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ABSTRACT

Background and Objectives: Increasing reports of Vancomycin creep have been observed in both Methicillin sensitive and Methicillin resistant staphylococcal isolates. The objective of this study was to determine the sensitivity pattern among clinical isolates of Methicillin sensitive and Methicillin resistant *Staphylococcus aureus* (MRSA)*, S. epidermidis, S. haemolyticus,* to Vancomycin, Teicoplanin and Linezolid. **Materials and Methods:** A total of 82 *S. aureus*, 34 *S. epidermidis* and 26 *S. haemolyticus* isolated during Jan 2011 to Dec 2011 were included in the study. None of the *S. aureus* isolates were resistant to Vancomycin and Linezolid. Only one MRSA isolate was resistant to Teicoplanin. Vancomycin resistant strains among Methicillin resistant *S. epidermidis* was one (4.3%) and among *S. haemolyticus* was two (8.3%) respectively. Teicoplanin resistant strains among Methicillin resistant *S. epidermidis* was six (26.1%) and among *S. haemolyticus* was two (8.3%). Linezolid resistant strains among Methicillin resistant *S. epidermidis* was two (8.7%) and among *S. haemolyticus* was one (4.2%). **Conclusion:** Emergence of Vancomycin, Teicoplanin and Linezolid resistance among Methicillin resistant coagulase negative staphylococci (CoNS) is alarming limiting the therapeutic options.

Key words: CoNS glycopeptides resistance, CoNS Linezolid, Teicoplanin resistance, CoNS Vancomycin, *S. epidermidis, S. aureus, S. haemolyticus*

INTRODUCTION

Increasing resistance to Vancomycin is a challenge in the management of *S. aureus* infections. Vancomycin creep has been observed in both Methicillin sensitive and Methicillin resistant staphylococcal isolates.[1] Among coagulase negative staphylococci it is commonly observed with *S. haemolyticus* & *S. epidermidis.*[2]

Though emergence of Vancomycin resistance is not a major problem in India, recent reports from Southern and Northern parts of India have documented the emergence of Vancomycin intermediate and resistant strains among staphylococcal isolates.[3-7] Data from the western part of India is scarce. It is postulated that the shift in Vancomycin MIC values may be associated with a concurrent rise in MIC values of other anti-MRSA agents. Hence we, analysed the data from our centre to find out the emergence of resistance to Vancomycin, Teicoplanin and Linezolid among Methicillin resistant and Methicillin sensitive staphylococal isolates.

Access this article online

Quick Response Code:

Website: www.jacmjournal.org

DOI: 10.4103/0972-1282.158766

MATERIALS AND METHODS

All *S. aureus, S. epidermidis and S. haemolyticus* isolated during Jan 2011 to Dec 2011 at Seven Hills Hospital, Mumbai, India were included. Only non repetitive clinical isolates were included in the study. This study was undertaken to determine the sensitivity pattern among clinical isolates of Methicillin sensitive and Methicillin resistant *S. aureus, S. epidermidis*, *S. haemolyticus* to Vancomycin, Teicoplanin and Linezolid.

Identification and susceptibility testing was done by automated Phoenix (Becton-Dickinson) system. *Staphylococcus aureus* ATCC 29213 was used as control strain. Minimum inhibitory concentration (MIC) interpretation criteria was as per Clinical Laboratory Standards Institute (CLSI) [Table 1a].[8]

RESULTS

A total of 142 staphylococci were isolated during the study period. Of these, there were 82 *S. aureus*, 34 *S. epidermidis*

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and 26 *S. haemolyticus* isolates from various clinical samples like pus, endotracheal secretions, catheter tip, blood and semen. Coagulase negative staphylococci represented either pathogen/colonisation. None of these isolates were part of surveillance screening. Distribution of MIC for different staphylococci is shown in [Table 1. Percentage susceptibility among Methicillin resistant and Methicillin sensitive staphylococci to Vancomycin, Teicoplanin, Linezolid is shown in Table 2.

A total of 23 (28.05%) isolates out of 82 *S. aureus* strains were MRSA. The Vancomycin MIC was ≤1 mg/L for 80 *S. aureus* isolates and only two isolates showed an MIC of 2 mg/L [Table 1b]. One isolate was from pus specimen of an inpatient and the other was a betalactamase producing isolate from a pus specimen of an outpatient which was also Teicoplanin resistant. Both the isolates were community acquired MRSA (CA-MRSA). None of the *S. aureus* isolates were resistant to Vancomycin [Table 3]. All the *S. aureus* isolates were sensitive to Linezolid.

Of the 34 *S. epidermidis* isolates, 23 (67.65%) were Methicillin resistant and 11 were Methicillin sensitive. Only one (0.03%) Methicillin resistant isolate was resistant to Vancomycin with an MIC >16 mg/L. This was also resistant to Teicoplanin and Linezolid. Teicoplanin resistance was seen in six isolates. MIC values of three isolates were not available. Only two (0.06%) Methicillin resistant isolates were resistant to Linezolid of which one was resistant to Vancomycin and had intermediate sensitivity to Teicoplanin. Among Methicillin sensitive isolates only one isolate had intermediate sensitivity to Teicoplanin with MIC 16 mg/L [Table 3].

Of the 26 *S. haemolyticus* isolates, 24 (92.31%) isolates were Methicillin resistant and two were Methicillin sensitive. Of the 24 Methicillin resistant isolates, two (0.08%) isolates were resistant to Vancomycin with MIC >16 mg/L and one had only intermediate sensitivity to Teicoplanin. There were two isolates resistant to Teicoplanin. Only one out of 24 (0.04%) isolates tested was resistant to Linezolid with MIC >4mg/L [Table 3].

DISCUSSION

After the emergence of Vancomycin-resistant enterococci in the 1980s, significant concern existed with regard to the potential for large outbreaks of Vancomycin-resistant *S. aureus* (VRSA) due to the acquisition of the *vanA* gene from enterococci. Fully Vancomycin-resistant strains of

Table 1a: MIC Interpretive criteria (mg/L)

Organism	Susceptible			Intermediate			Resistant		
	Vancomycin	Teicoplanin	Linezolid	Vancomycin	Teicoplanin	Linezolid	Vancomycin	Teicoplanin	Linezolid
S.aureus	≤2mg/L	≤8mg/L	≤ 4mg/L	4-8 mg/L	16 mg/L	—	>=16 mg/L	>= 32mg/L	>=8mg/L
CONS	≤4mg/L			8-16 mg/L			>=32 mg/L		

Table b: Distribution of MIC (mg/L)

Number Of isolates	Vancomycin					Teicoplanin					Linezolid					
	≤1	2	4	16	>16	<1	2	4	8	16	>16	≤0.5	1	2	4	>4
S.aureus (82)	80	2		—	—	77	1	3	—	—	1	—	47	33	2	—
S.epidermidis (34)	26	6	1	—	1	—	6	12	4	3	6	—	7	15	9	2
S.haemolyticus (26)	18	5	—	1	2	1	1	10	8	4	2	1	22	—	—	1

Note: Out of 34 *S.epidermidis* only 31 isolates were tested for Teicoplanin and 33 were tested for Linezolid. Out of 26 *S.haemolyitcus* only 24 isolates were tested for Linezolid sensitivity.

Table 2: Vancomycin, Teicoplanin, Linezolid percent susceptibility

Antibiotics	Isolates	Methicillin resistant			Methicillin sensitive		
		Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
Vancomycin	*S.aureus* (82)	100 (23/23)	—	—	100 (59/59)	—	—
	S.epidermidis (34)	95.7 (22/23)	—	4.3 (1/23)	100 (11/11)	—	—
	S.haemolyticus (26)	87.5 (21/24)	4.1 (1/24)	8.3 (2/24)	100 (2/2)	—	—
Teicoplanin	*S.aureus* (82)	95.66 (22/23)	—	4.3 (1/23)	100 (59/59)	—	—
	S.epidermidis (34)	60.9 (14/23)	13 (3/23)	26.1 (6/23)	90.9 (10/11)	9.1 (1/11)	—
	S.haemolyticus (26)	87.5 (21/24)	4.2 (1/24)	8.3 (2/24)	100 (2/2)	—	—
Linezolid	*S.aureus* (82)	100 (23/23)	—	—	100 (59/59)	—	—
	S.epidermidis (34)	91.3 (21/23)	—	8.7 (2/23)	100 (11/11)	—	—
	S.haemolyticus (26)	95.8 (23/24)	—	4.2 (1/24)	100 (2/2)	—	—

Table 3: Distribution of Methicillin resistant isolates among the various specimens and their MIC to Vancomycin, Teicoplanin and Linezolid

Isolate	Specimen	Vancomycin MIC(S/I/R)	Teicoplanin MIC(S/I/R)	Linezolid MIC(S/I/R)
MRSA (23/82)	Pus	2 (S)	>16 (R)	4 (S)
MRSE (23/34)	Pus	>16 (R)	>16 (R)	>4 (R)
	Blood	2 (S)	16 (I)	>4 (R)
	ET secretion	≤1 (S)	>16 (R)	4 (S)
	ET secretion	2 (S)	>16 (R)	4 (S)
	ET secretion	≤1 (S)	16 (I)	2 (S)
	Blood	4 (S)	>16 (R)	2 (S)
	Blood	≤1 (S)	>16 (R)	2 (S)
	Catheter tip	≤1 (S)	>16 (R)	4 (S)
MRSH (24/26)	Semen	16 (I)	16 (I)	1 (S)
	Pus	>16 (R)	>16 (R)	>4 (R)
	Blood	≤1 (S)	16 (I)	2 (S)
	Blood	≤1 (S)	16 (I)	1 (S)
	ET secretion	2 (S)	16 (I)	1 (S)
	Catheter tip	>16 (R)	>16 (R)	1 (S)

S: Sensitive; I: Intermediate; R: Resistant

S. aureus (VRSA) due to the acquisition of the *vanA* gene from Vancomycin-resistant enterococci were first reported from the United States in 2002.[9]

In our study Vancomycin resistance was not noted among *S. aureus* isolates. In our study, only two (2.4%) *S. aureus* isolates showed increase in MIC for Vancomycin and one (1.2%) was MRSA, while other was MSSA. Though this constitutes a very small percentage, close monitoring to note the creep in MIC is warranted due to therapeutic implications. hVISA (heteroresistant VISA) is defined as a Vancomycin susceptible *S. aureus* (VSSA) strain with MIC ≤ 2 on routine testing, that upon subculture produces sub-colonies with MIC in the VISA/VRSA range at the frequency of ≥1x10^6 according to population analysis profile (PAP). Though we did not note a creep in the Vancomycin MIC value, hVISA prevalence was not looked into and this was a limitation to our study. However, it is difficult to confirm the presence of heteroresistance in clinical practice because conventional microbiological methods, including MIC determination, cannot detect hVISA. Therefore, if hVISA infection is strongly suspected based on clinical features, population analysis has to be performed at a reference laboratory. Heteroresistance has also been observed in coagulase-negative staphylococci.[10]

Elevated Vancomycin MIC in *S. aureus* has been associated with increased mortality in patients with Methicillin-susceptible *S. aureus* infections when they are treated with either Vancomycin or Flucloxacillin. Based on neutropenic mouse models, in vitro studies and limited data from human studies, the AUC/MIC ratio has been used as a preferred parameter for measuring the effectiveness of Vancomycin in treating *S. aureus* infections. A specific AUC/MIC threshold of 400 has been advocated as a target to achieve clinical effectiveness with Vancomycin, based on the initial clinical data from pneumonia and more recent data from bacteremia. Using Monte Carlo simulations, it has been suggested that the probability of attaining this ratio is approximately 100% in isolates with MIC ≤ 0.5 mg/L and the probability falls to 0% in isolates with MIC of 2 mg/L. Based on simulated models, it has further been reported that a daily dose of 3-4 gm of Vancomycin will be required to provide 90% probability of attaining the target AUC/MIC of 400 for an isolate with MIC 1 mg/L. One needs to consider alternate antibiotics for MRSA isolates especially for respiratory isolates if MIC >1mg/L.[1] In our study both the isolates with MIC 2 mg/L were from non respiratory specimens. Only one MRSA isolate was resistant to Teicoplanin indicating low prevalence of resistance and all isolates were sensitive to Linezolid.

Coagulase-negative staphylococci (CoNS), especially *Staphylococcus epidermidis*, are major nosocomial pathogens causing a variety of device-related infections in humans. Infections caused by coagulase negative staphylococci are mainly from indwelling foreign bodies, and their role as agents of osteomyelitis following cardiothoracic, ophthalmic and neurosurgical procedures and in immunocompromised patients have been well established.

Unlike that of *Staphylococcus aureus,* CoNS resistance to glycopeptides applies almost exclusively to Teicoplanin. The mechanisms involved are unclear, but CoNS with decreased susceptibility to glycopeptides show cell wall thickening and tend to form cellular aggregates.[9] In our study Vancomycin, Teicoplanin and Linezolid resistance was noted among Methicillin resistant CoNS. Reports on Teicoplanin susceptibility among CONS are scarce from India.[10-12] In our study Teicoplanin resistance that was limited to Methicillin resistant isolates among CoNS is consistent with other reports. There have been reports of *S. haemolyticus* resistant to Teicoplanin but sensitive to Vancomycin. Similar findings were observed in our study.[13,14] Two pus isolates were resistant to Vancomycin, Teicoplanin and Linezolid. Both these isolates showed sensitivity to Tetracycline and Trimethoprim-Sulphamethaxazole. The appropriate antibiotics were selected based on antibiogram to treat such patients.

Recent epidemiological data show that Linezolid resistance occurs in ≤1% of *S. aureus* isolates and ≤0.1% of CoNS in the US. Staphylococcal Linezolid resistance is rare from India and is limited to case reports and is observed

only among CoNS isolates. In our study also, Linezolid resistance that was observed among Methicillin resistant CoNS is consistent with Indian reports.[10-12]

CONCLUSION

Prevalence of Vancomycin, Teicoplanin and Linezolid resistance among *S. aureus* isolates has not yet been detected in isolates from our hospital and no such reports are available from the western part of India. Vancomycin resistant coagulase negative staphyloccal isolates are also resistant to Teicoplanin and associated with a creep in Linezolid MICs, limiting the therapeutic options for CoNS infections. Though coagulase negative staphylococcal infections are limited to an immunocompromised host, emergence of Vancomycin, Teicoplanin and Linezolid resistance among Methicillin resistant coagulase negative staphylococci is alarming as this can spread to coagulase positive staphylococci in the future, limiting the therapeutic options. The only way out is to rationalise the use of these antibiotics.

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How to cite this article: Shivaprakasha S. Determination of Vancomycin, Teicoplanin and Linezolid resistance among Staphylococcal isolates from a tertiary care hospital. J Acad Clin Microbiol 2015;17:3-6.

Source of Support: Nil. **Conflict of Interest:** None declared.