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Original Article

Microbiology Section

Incidence of Vancomycin Resistant *Staphylococci* from Various Clinical Isolates In a Tertiary Care Hospital

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ABSTRACT

Vancomycin, a glycopeptide antibiotic, is the main antimicrobial agent available to treat life-threatening infections with Methicillin resistant Staphylococcus. 500 Staphylococcal isolates were speciated by conventional biochemical tests, as S.aureus and Coagulase negative Staphylococci (CONS). Isolates were screened for Vancomycin resistance by disc diffusion, inoculation on vancomycin screen agar & confirmed by E test. Vancomycin resistant isolates were tested for their susceptibility to Linezolid & Quinupristine-dalfopristine using the disc diffusion method. 294/500 isolates were S.aureus of which 155 were MRSA & 206/500 were CONS out of which 122 were MR-CONS. Of the 3 vancomycin resistant isolates, one was VRSA and 2 were VRCONS, which were sensitive to linezolid, quinupristin/ dalfopristin.

Key Words: S.aureus, VRSA, VRCONS, Vancomycin screen agar

INTRODUCTION

Staphylococcus spp. is one of the most common causes of nosocomial and community-acquired infections, with high rates of mortality.

S. aureus has a proclivity for the acquisition of resistance elements to a wide range of antibiotic classes. At present, the glycopeptide antibiotic, vancomycin, is often the sole remaining antibiotic effective against S. aureus infections.

Infections caused by less virulent Coagulase-*Negative Staphylococci* (CONS) with reduced susceptibility to vancomycin also been reported [1].

Vancomycin resistance is acquired by mutation and thickening of cell wall due to accumulation of excess amounts of peptidoglycan.

The prototype vanA gene is present on Transposon Tn1546, a mobile genetic element containing genes responsible for high-level glycopeptide resistance among Enterococci and several other gram-positive organisms [2]. Acquisition of vancomycin resistance usually involves horizontal transfer of a Tn1546-containing plasmid & this phenomenon was successfully accomplished in the laboratory [3].

Hetero Vancomycin resistant *Staphylococcus aureus* (Hetero VRSA) is an evolutionary status between Vancomycin susceptible *Staphylococcus aureus* (VSSA) & Vancomycin resistant *Staphylococcus aureus* (VRSA). It produces VRSA cells at a high frequency to secure its survival as a strain. These VRSA cells may return to hetero-VRSA status when the pressure is lifted [4].

There are 2 types of hetero-VRSA: stable and unstable. The stable variant of hetero-VRSA may be established either by cycles of exposure to vancomycin or by one-step stable genetic alteration. Many unstable hetero-VRSA strains are produced in the clinical setting. These strains express hetero-resistance directly after isolation from patients who undergo Glycopeptide therapy, but they lose their resistance during strain storage in drug-free media [4].

MATERIALS AND METHODS

A total 500 clinical isolates of Staphylococcal species isolated from clinical samples received at Diagnostic Microbiology Section of Microbiology Department, of tertiary care Hospital & Medical College were included in the study.

Species identification: The Staphylococcal isolates were speciated by conventional biochemical tests, as S.aureus and *Coagulase negative Staphylococci* (CONS) .

Disc Diffusion [5]

AST of the isolates was done against Penicillin (10 U), Amikacin (30µg), Ofloxacin (10 µg), Ciprofloxacin (5µg), Chloramphenicol (30µg), Erythromycin(15µg), Roxithromycin (15µg),

Cephazolin (30µg), Ceftriaxone(30µg), Vancomycin(30 µg)(Hi media - india), by Kirby-Bauer disc diffusion method [5]. S. aureus ATCC 29213 was used as Q.C. strain. Vancomycin resistant isolates were tested for their susceptibility to Linezolid (30 µg) & Quinupristine-dalfopristine (15 µg) using the disc diffusion method.

Determination of Methicillin / Oxacillin Resistance by disc diffusion:

All 500 Staphylococcal isolates were screened for methicillin resistance using Oxacillin disc (1µg) on MHA with 4% NaCl, as. Plates were incubated for 24 hours at 35ºC.

Agar screen for Vancomycin resistance [6]

The 277 MR Staphylococcal isolates were further tested for vancomycin resistance using the vancomycin screen agar. Brain Heart Infusion agar (Hi – media, India) was incorporated with 6 µg/ml vancomycin were used. Inoculums of 0.5 McFarland standard turbidity were prepared. The BHI screen agar plates were spot inoculated with 10 µl of the culture suspension and was incubated for 24 hours at 35°C in ambient air. Any visible growth of an isolate on vancomycin screen agar (VSA) was considered to be resistant to vancomycin.

Determination of Minimum Inhibitory Concentration of Vancomycin:

MIC of vancomycin was determined by vancomycin E test (Himedia, India) for the 3 Staphylococcal isolates that showed vancomycin resistance by the disc diffusion and on the screen agar. *S. aureus* ATCC 29213 and Enterococcus faecalis ATCC 29212 were used as vancomycin susceptible Q.C. strains and E. faecalis ATCC 51299 as vancomycin resistant Q.C. strain.

RESULTS

AST results of all Q.C. strains were within acceptable limits.

Out of 500 Staphylococcal isolates, 294 isolates were identified as *S.aureus* and 206 isolates were CONS. Methicillin resistance was seen in 277 Staphylococcal isolates, of which 155 were MRSA & 122 were MRCONS. Of all staphylococcus isolates 3 were vancomycin resistant of which one was MRSA and 2 were MRCONS. These three isolates were sensitive to linezolid, quinupristin/ dalfopristin. MIC to vancomycin was 1024µg/ml in all the 3 isolates. 16% of *S. aureus* & 15% of CONS are MDR and sensitive to vancomycin only.

DISCUSSION

Vancomycin, a glycopeptide antibiotic, is currently the main antimicrobial agent available to treat life-threatening infections with MRSA.

VRSA isolate detected in our study was isolated from the Pus of a 58-years old patient who was admitted in the surgical ward and was on vancomycin therapy; the other 2 vancomycin resistant CONS were isolated from blood of two neonates admitted to the PICU of our hospital. [Table/Fig-1].

Sample	S. aureus			CONS		
	No. of isolates	MRSA	VRSA	No. of isolates	MR CONS	VR CONS
Wound cs	88	57	Nil	22	14	Nil
Sputum cs	9	10	Nil	Nil	Nil	Nil
Blood cs	149	71	Nil	166	102	2
Pus/ Drain cs	34	14	1	8	3	Nil
Ocular discharge/ Corneal scrapping	4	1	Nil	3	Nil	Nil
Urine cs	9	2	Nil	5	3	Nil
Body fluid cs	1	Nil	Nil	2	Nil	Nil

[Table/Fig-1]: Sample wise incidence of Methicilin & Vancomycin resistant isolates in present study

The first MRSA to acquire resistance to vancomycin (Vancomycin intermediate *Staphylococcus aureus* – VISA), was isolated from a Japanese patient in 1996 [7] & first report of hetero Vancomycin intermediate *Staphylococcus aureus* (hVISA) was reported from Australia in 2001 [8]. A few other countries have also reported the reduced susceptibility of S. aureus and CONS against glycopeptides [9].

First case of VRSA was reported in 2002 in USA [10]. Subsequently several vancomycin resistant S aureus (VRSA) strains were isolated from USA, France, Korea, South Africa, and Brazil [4]. So far, only nine cases of VRSA have been reported from the United States, with two additional cases, one from India and other from Iran [11].

The first VRSA isolate (MIC 64 µg/ml) was reported from Kolkata hospital, India, in June 2005 [12] Later, a 0.2%, prevalence of VRSA was documented from Varanasi, India in 2008 [7] which was similar to our study. The Prevalence of VISA in these studies was about 0.7%, though in the present study no VISA were found. Recently reported cases of VRSA from Jordan, VRCONS from Brazil & VISA from India were reviewed

by Hare Krishna Tiwari et al., in their study [9].

The VRSA isolate of our study was probably heteroresistant, as it lost its resistance & became susceptible to vancomycin, on serial subculture in drug free media. Heteroresistance can be detected by Population analysis (Pap method) [13]. However, this assay could not be performed due to certain constraints.

Conventional susceptibility tests (MIC, disk diffusion tests) cannot discriminate between VSSA and heteroVRSA [4]. However, the two may behave in a significantly different manner in response to vancomycin therapy which is important in determing patient response to tretment [4].

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