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In Vitro Activity of Vancomycin and Teicoplanin Against Coagulase Negative Staphylococci

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Abstract

Objectives: With the increase in nosocomial infections caused by coagulase negative staphylococci (CNS), laboratory diagnosis of CNS with reduced susceptibility to glycopeptides (vancomycin and teicoplanin) has become important. This study was designed to determine the glycopeptide susceptibility of clinical isolates of methicillin resistant coagulase negative staphylococci (MRCNS) at the department of microbiology, government medical college and hospital, Amritsar, India.

Methods: A total of 250 CNS isolated from various clinical specimens were speciated and their methicillin resistance was detected by studying the minimum inhibitory concentration (MIC) of oxacillin by macrobroth dilution method. Glycopeptide susceptibility of 130 methicillin resistant strains obtained was determined for vancomycin by vancomycin screen agar test, MIC of vancomycin by macrobroth dilution/and E test. Teicoplanin susceptibility was determined using teicoplanin disc diffusion test and MIC was determined by macrobroth dilution method.

Results: All the MRCNS isolates were found to be susceptible to vancomycin and teicoplanin. MIC of vancomycin ranged between ≤0.5 µg/ml to 1 µg/ml and of teicoplanin from ≤0.5 µg/ml to 2µg/ml.

Conclusion: Continuous monitoring of MIC of vancomycin in MRCNS is required to prevent the emergence of vancomycin resistance in these multidrug resistant organisms.

Keywords: Glycopeptides; Minimum inhibitory concentration.

Introduction

The treatment of infections caused by coagulase negative staphylococci (CNS) is complicated by the unique ability of these organisms to express resistance to multiple antibiotics.[1] Resistance to methicillin is almost universal among the isolates recovered from hospitalized patients. For this reason, glycopeptide antibiotics (vancomycin and teicoplanin) have now become the foundation of chemotherapy in CNS infections worldwide.[2,3] However, in recent years, decreased susceptibility of these isolates to glycopeptides has been reported from different parts of the world including India.[2-4] This poses a fearsome threat to the already challenging therapy of methicillin resistant coagulase negative staphylococci (MRCNS). Therefore, the present study was undertaken to determine the glycopeptide susceptibility of clinical isolates of MRCNS at the department of microbiology, government medical college and hospital, Amritsar, India

Methods

A total of 250 consecutive, non-repeat CNS isolates obtained from blood (112), pus (73), urine (57), intravenous catheter tips (6), ascitic (1) and synovial (1) fluids from January 2008 to July 2009 from inpatients, at the department of microbiology, government medical college and hospital, Amritsar, India. The isolates studied had the potential of being clinically significant on the basis of source and/or amount of organisms isolated. The strains isolated from blood, other sterile body fluids (Sole organism or in moderate to heavy amounts), urine with significant colony counts (Greater than 10^5 organisms per ml) and catheter tips (Greater than 15 colonies) were included in the study. After their identification and speciation according to the methodology suggested by Kloos and Schleifer,[5] the isolates were subjected to antibiotic sensitivity testing by Kirby Bauer Disc diffusion method.

Methicillin resistance was determined by studying the minimum inhibitory concentration (MIC) of oxacillin (HI media, Mumbai) by macrobroth dilution method (dilutions ranging from 0.25 µg/ml - 256 µg/ml).[2,5,6] For the detection of resistance to vancomycin, the methods used included; a) vancomycin screen agar test using 6 µg/ml of vancomycin incorporated in Brain Heart infusion agar, and b) MIC of vancomycin (VHB Life Sciences) by macrobroth dilution method with dilutions ranging from 0.5 µg/ml-128 µg/ml.[2,6,7] The MIC values obtained were cross checked by E test (Hi Comb MIC test, HI Media). The E test was performed according to the protocol supplied by the manufacturer and MIC was calculated as the value at which the zone of inhibition converged on the comb like projection of the strip.[8] In disc diffusion test for teicoplanin, (30 µg) discs were used and MIC of teicoplanin (Lupin) was determined by macrobroth dilution method with dilutions ranging from 0.5-128 µg/ml. The results were interpreted as per CLSI guidelines.[6] *S. aureus* ATCC 29213 was used as a reference strain.

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Results

Of the 250 isolates, 130 (52%) were found to be resistant to methicillin with MIC ≥0.5 µg/ml (0.5-32 µg/ml) by the broth macrodilution method. Out of 130 MRCNS, the majority were *S. epidermidis*, amounting to 49.23% (64/130), followed by *S. haemolyticus* which accounted for 43.07% (56/130), and then *S. warneri* accounting for 3.07% (4/130), while *S. saprophyticus* and *S. cohnii* accounted for 2.3% (3/130) each. The MIC for vancomycin and teicoplanin in the studied isolates are given in Tables 1 and 2 respectively.

Table 1: MIC of Vancomycin for various species of MRCNS and MSCNS by Macrobroth dilution Method.

Species of CNS	MRCNS (n=130)		MSCNS (n=120)	
	MIC≤0.5µg/ml	MIC=1µg/ml	MIC≤0.5µg/ml	MIC=1µg/ml
S. epidermidis (n=137)	59	5	71	2
S. haemolyticus (n=100)	46	10	41	3
S. saprophyticus (n=5)	3	-	2	-
S. warneri (n=5)	2	2	1	-
S. cohnii (n=3)	2	1	-	-
Total	112	18	115	5

p=0.008 significant

CNS= Coagulase negative staphylococci, MRCNS=Methicillin resistant coagulase negative staphylococci, MSCNS= Methicillin sensitive coagulase negative staphylococci, MIC=Minimum inhibitory concentration

Table 2: MIC of Teicoplanin from various species of MRCNS and MSCNS by Macrobroth dilution Method.

Species of CNS	MRCNS (n=130)			MSCNS (n=120)		
	MIC ≤0.5µg/ml	MIC =1µg/ml	MIC =2µg/ml	MIC ≤0.5µg/ml	MIC =1µg/ml	MIC =2µg/ml
S. epidermidis (n=137)	54	7	3	64	9	0
S. haemolyticus (n=100)	31	16	9	35	9	0
S. saprophyticus (n=5)	3	0	0	1	1	0
S. warneri (n=5)	4	0	0	1	0	0
S. cohnii (n=3)	3	0	0	0	0	0
Total	95	23	12	101	19	0

p=0.04 significant

CNS= Coagulase negative staphylococci, MRCNS=Methicillin resistant coagulase negative staphylococci, MSCNS= Methicillin sensitive coagulase negative staphylococci, MIC=Minimum inhibitory concentration

All the 130 MRCNS isolates were found to be susceptible to vancomycin as they did not show any growth on vancomycin screen agar. The MIC values of all the isolates for vancomycin by macrobroth dilution method were in the susceptible range (breakpoint ≤4 µg/ml) and 86.15% (112/130) exhibited MIC ≤0.5 µg/ml. E test for vancomycin corroborated the results obtained by macrobroth dilution method. All the 130 MRCNS were also found to be sensitive to teicoplanin in both disc diffusion method and macrobroth dilution, with MIC ranging from ≤0.5 µg/ml - 2µg/ml. Further analysis of MIC values of vancomycin (1 µg/ml) and teicoplanin (1,2 µg/ml) in comparison to MIC value of ≤0.5 µg/ml; was found to be statistically significant for MRCNS compared to MSCNS. ($p<0.05$)

Discussion

Although a large number of studies have reported uniform susceptibility of CNS to vancomycin and teicoplanin similar to that of our study,[9-11] there are reports of decreased susceptibility to frank resistance to vancomycin amongst CNS from Japan, Russia, United Kingdom, USA, Brazil and France and India.[2,4,8] From North India, Tiwari et al. reported isolation of one strain of CNS (*S. epidermidis*) resistant to vancomycin and two CNS strains (*S. epidermidis*) intermediate to vancomycin.[2]

Recently, five vancomycin intermediate CNS (MIC≥5 µg/ml) strains were isolated from South India too.[8] Reduced susceptibility of

CNS isolates (*S. haemolyticus*) to teicoplanin has been reported by Chaudary et al.[3] Also, 100% susceptibility of CNS to vancomycin and teicoplanin in the current study could be due to the preferential use of linezolid by clinicians in our hospital; and glycopeptides are not uniformly administered to treat infections caused by MRCNS. The emergence of resistance may be as a result increasing selective pressure of vancomycin due to its widespread use to treat infections caused by staphylococci (methicillin resistant isolates; in particular) and other gram-positive cocci.[2,8] This is a matter of great concern because there are currently no other alternative treatment options available in India.

Conclusion

Overall, the results from this study emphasize that the emergence of vancomycin resistance in CNS could be limited by promoting the judicious use of the drug by discouraging its overuse and misuse. Nationwide surveillance programs should be conducted to map out the glycopeptide susceptibility pattern of CNS in India. In order to achieve this, all laboratories should have effective methods of detecting vancomycin resistance which could be expressed heterogeneously. Though the results of vancomycin screen agar test in our study correlated with MIC values of vancomycin by macrobroth dilution and E test, making the dilutions can be difficult for the macrobroth dilution test. All the methicillin resistant coagulase negative staphylococcal isolates could be screened by vancomycin screen agar test and only those positive by this method should be further tested for vancomycin MIC.

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