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Microbial Surveillance and Susceptibility of Gram-Positive Bacteria to Antibiotic Drugs

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Abstract: *Incidence of antimicrobial resistance among Gram-positive organisms has been increasing steadily to most of the currently available anti bacterials, making it extremely difficult to treat infections. Purpose of this study was to assess the epidemiology of infections caused by multi-drug resistant (MDR) Gram positive isolates in India and to survey response of antimicrobial agents to these strains. This study involved 408 Gram positive isolates including S. aureus (211), Methicillin-resistant Staphylococcus aureus (MRSA) (130), Staphylococcus epidermidis (15), Streptococcus pneumoniae (12), Streptococcus pyogenes (13), Streptococcus bovis (7), Streptococcus agalactiae (9) and Enterococcus faecalis (11) which were collected from different parts of India. Susceptibility study was performed by broth microdilution method as recommended by Clinical and laboratory standard institutes (CLSI). Our study revealed that Vancoplus is the most effective with > 90 % susceptibility to most of the pathogens like S. aureus, MRSA and S. epidermidis with MICs 0.0625-2 µg/ml followed by linezolid with ≤ 85 % susceptibility to the said pathogens with MICs 1-4 µg/ml. The susceptibility of other drugs varied between 19 to 84 %. Among streptococcus, the susceptibility of Vancoplus varied between 91 to 100% with MICs 0.3125 to 1 µg/ml whereas linezolid showed 66 to 84 % susceptibility with MICs 0.5-4 µg/ml. The susceptibility of other drugs ranged between 23 to 71 %. About 90.9 % E. faecalis isolates were susceptible to Vancoplus at 0.0625-4 µg/ml compared to 81.8% to Linezolid, around 72% to vancomycin, daptomycin and teicoplanin and only 27.3% to clindamycin In conclusion, Vancoplus demonstrated potent in vitro activity against Gram-positive staphylococcal, streptococcal and enterococcal isolates. The results of this surveillance study can serve as a benchmark for monitoring the in vitro activity of this new agent.*

Keywords: *Clinical isolates, resistance, susceptibility, Vancoplus*.

I.Introduction

A number of Gram positive species known to cause disease in humans include methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), vancomycin-intermediate and resistant *S. aureus* (VISA and VRSA), coagulase-negative *staphylococcus* (CONS), penicillin-resistant *Streptococcus pneumoniae*, *Enterococcus faecalis*, *Staphylococcus epidermidis*, *Streptococcus spp.* β-Hemolytic Group (*S. pyogenes* and *S. agalatiae*) and Streptococcus spp. Viridans Group (*S. bovis*) which are the most common pathogens showing increased resistance to many antibiotics [1-3].

In last two decades, these Gram-positive pathogens have raised serious medical concerns as severity of infections caused by these organisms represent a major public health burden by increased morbidity and mortality, increased expenditure on patient management and implementation of infection control measures [4]. Gram-positive bacteria accounts for more than 50% of all bloodstream infections [5]. Besides, they also cause meningitis, endocarditis, osteomyelitis, septic arthritis, toxic shock syndrome and food poisoning [3,6-10]. Clark et al. [11] conducted surveillance studies in intensive care units and demonstrated that Gram-positive organisms such as S. aureus, coagulase-negative staphylococci and enterococci are among the most common bacteria infecting patients in intensive care units (ICUs).

Despite improvements in immunization, infection control policies and medical practice, the rate of emergence of resistance against these strains has continued to rise through various mechanisms which is worrisome. Gram-positive pathogens, staphylococci, enterococci and streptococci with methicillin resistant MRSA, VRSA and VRE are getting resistant to commonly used drugs such as methicillin, oxacillin and nafcillin, macrolides, tetracycline and aminoglycosides offering the greatest challenge to health care worldwide [12-14].

A number of recent studies showed that vancomycin treatment failure rate has exceeded 40% [15]. Several reports from India recorded the emergence of various degree of vancomycin resistance [13,16] along with other parts of the world including France [17], United Kingdom [18] and Korea [19]. Genus enterococcus shows resistance not only to glycopeptides, β-lactams and fluoroquinolones, but also demonstrate high levels of resistance to aminoglycosides (gentamicin and streptomycin), leading to drastically reduced therapeutic options for patients infected with these bacteria and regarded as important pathogens with clinical relevance [20].

Resistance to glycopeptides first reported in Japan [21], was later observed in many other countries including U.S. [22-23]. The emergence of glycopeptide-resistant Enterococci and Staphylococci, underline the

need for therapeutic alternatives. There is a paucity of information on drug resistance in Gram positive organisms in our country. This study was performed to determine the prevalence of resistance among Gram positive isolates in India and to survey response of antimicrobial agents under surveillance programme.

II.Materials And Methods

2.1. Bacterial strains

Present survey was conducted by Emerging Antimicrobial Resistance Society (EARS, non-governmental organization (NGO) and the study was conducted by Venus Medicine Research Centre, Himachal Pradesh, India from January 2012 to November 2014. The objective was to check the antibiotic susceptibility of Gram positive organisms collected from various parts of India with the help of emerging antimicrobial resistance society (EARS). A total of 408 Gram positive isolates collected from various hospitals of India were included in this study. The pool comprised of *S. aureus* (211), MRSA (130), *S. epidermidis* (15), *S. pneumoniae* (12), *S. pyogenes* (13), *S. bovis* (7), *S. agalactiae* (9) and *E. faecalis* (11). All strains were subcultured on Mueller Hinton (Himedia, Mumbai, India) agar plates with 5% sheep blood (BBL). The plates were incubated 18 to 24 hr prior to testing.

2.2. Antibacterial agents

Antibacterial agents included for susceptibility testing were Vancoplus (a novel antibiotic adjuvant entity ceftriaxone sodium and vancomycin hydrocloride with VRP1020), teicoplanin, linezolid, daptomycin and clindamycin. All the drugs were reconstituted in water for injection except Vancoplus which was reconstituted in solvent provided with the pack as per manufacturer's instructions. Working solutions were prepared using Mueller Hinton broth (MHB, Himedia, Mumbai, India), and serial two fold dilutions were made using Cation-Adjusted Mueller-Hinton broth (CAMH, Himedia, Bombay, India) in wells of 96-well plate.

2.3. Minimum inhibitory concentration (MIC) testing

Minimum inhibitory concentrations were performed by broth microdilution method with a final inoculum of 10^6 cfu/ml, as recommended by Clinical and laboratory standard institutes (CLSI) [24]. Serial dilutions of the antibiotics ranging from 0.0156-1024 µg/ml were prepared and used on the same day. MIC was defined as the lowest concentration of drug which inhibited visible growth of bacteria. Results were interpreted according to CLSI (Clinical and Laboratory Standards Institute) and EUCAST (European Committee on Antimicrobial Susceptibility Testing).

III.Results

3.1. Bacterial strains

In current investigation, a total of 408 organisms were included out of which 87.2 % were staph infections where 83.5% strains were of coagulase +ve staphylococci *S. aureus* (211) , MRSA (130), 3.6% were coagulase negative staphylococci (*S. epidermidis*, 15), 12.7 % were streptococci where 2.9% were α hemolytic (*S. pneumoniae*, 12), 5.3% were β haemolytic (*S. pyogenes*, 13, *S. agalactiae* 9) and remaining 4.4% were γ haemolytic (*S. bovis* 7, *E. faecalis*, 11) (Table 1).

3.2. Antibiotic susceptibility results

In vitro activities of the tested drugs against Gram positive strains are summarized in Tables 2 and 3. Our data demonstrated that Vancoplus appeared to be the most effective among tested drugs. More than 90% susceptibility was observed to Vancoplus (MIC 0.0625-2 µg/ml) in staphylococcal isolates, 95.7 % *S. aureus*, 90% MRSA and 93.3% *S. epidermidis* and only 4.3 to 6.6% isolates were resistant to Vancoplus (MIC 16-256 µg/ml). Second most active agent was Linezolid which remains susceptible against ≤85% of Staphylococcus (MIC 1-4 µg/ml), with resistance identified in 15.2% of *S. aureus*, 29.3% MRSA and 40% of *S. epidermidis*. Daptomycin was the third most active drug which exhibited ≤80% susceptibility against Staphylococcus (MIC 0.0312-1 µg/ml), with resistance identified in 20.4 % of *S. aureus*, 73.9 % MRSA and 53.4 % of *S. epidermidis*. A similar trend was observed for vancomycin. Although teicolpnain exhibited slightly better susceptibility to MRSA (43.8%) in comparison to daptomycin, but the response to other pathogens was identical (MIC 1-8 µg/ml). Clindamycin was observed to be least susceptible showing only 51.6, 19.2 and 33.3 % susceptibility against *S. aureus*, MRSA and *S. epidermidis* at MIC 0.0625-0.5 µg/ml.

Among streptococcus, 91.6 % *S. pneumoniae* and 100 % isolates of each *S. pyogenes*, *S. bovis* and *S. agalactiae* were found to be susceptible to Vancoplus at MIC 0.03125 to 1 µg/ml. A 20-30% lesser susceptibility was observed with linezolid (MIC 0.5-4 µg/ml). Teicoplanin appeared to be the third best drug to streptococci after Vancoplus and Linezolid. Daptomycin was >40% resistant to all strains where as clindamycin was >60% resistant. Vancomycin exhibited 88.8 % susceptibility to *S. agalactiae*, but was found to be ≤50% susceptible to other strains of this class. About 90.9% E. faecalis isolates were susceptible to Vancoplus at 0.0625-4 µg/ml.

Susceptibilities of linezolid and clindamycin against the same strain were 81.8 and 27.3 % at 0.0625-2 and 0.0625-0.25 µg/ml. For vancomycin, daptomycin and teicoplanin, approximately 72.7 % isolates of E. faecalis isolates were found to be susceptible to these drugs.

IV.Discussion

A number of studies have shown the changing trends of antimicrobial resistance among Gram-positive organisms to several antimicrobials during the past several years [25-27]. According to Centers for Disease Control and Prevention (CDC) statistics, more than 70% of bacteria causing hospital-acquired infections found to be resistant to at least 1 of the antibiotics most commonly used to treat them. In the past few years, increasing rates of vancomycin resistance has been reported in enterococci [28], vancomycin tolerence in S. pneumoniae [29] and S. aureus with reduced susceptibility of full resistance [26].

This was the first surveillance study of the activity of Vancoplus, a novel antibiotic adjuvant entity, against gram-positive clinical isolates from India. Approximately 408 isolates collected represent a diverse geographic and patient population and were obtained from clinically relevant infections. In this study, *S. aureus* was the most frequent gram-positive bacterium included in the current study. Our study showed that less than 5 % of *S. aureus* isolates were resistant to Vancoplus whereas other drugs demonstrated 15 to 48 % resistance to the same isolates. Methicillin was introduced in clinical use in 1960 since then methicillin resistant *S. aureus* have been reported various parts of the world [30-32]. We found that 4.6% MRSA were resistant to Vancoplus while 26 to 80 % resistance observed for other drugs. The susceptibility of MRSA to vancomycin may be declining and reports of treatment failures are increasing [33-37]. The varied level of vancomycin resistance was reported from different parts of the world [13,16,38-39].

The most common mechanisms of Staphylococcus for linezolid resistance is mutation (G2576T) to the 23S rRNA or the presence of a transmissible cfr ribosomal methyltransferase [40]. In our study, among streptococci, only 8.3 % of *S. pneumoniae* were resistant to Vancoplus whereas none of the isolates of *S. pyogens, S. bovis* and *S. agalactiae* was resistant to Vancoplus. *E. faecalis* was most susceptible to Vancoplus whereas other drugs found to be highly resistant. Average susceptibility of Vancoplus to staphylocci was 93% as against second best drug linazolid 71.8% and in Streptococci, Vancoplus average susceptibility was 96.5% as against 76.4% with Linezolid.

Overall, Vancoplus (combination of vancomycin plus ceftriaxone alongwith VRP1020) demonstrated potent in vitro activity against collected Gram positive isolates of staphylococci and streptococci including those resistant to other antimicrobial agents. MIC of Vancoplus was found to be lower than those of other comparator drugs. The enhanced activity of Vancoplus to these isolates may be due to synergestic action of ceftriaxone, vancomycin and VRP1020 (a non antibiotic adjuvant which prevents degaradtion of antibiotics). Ceftriaxone inhibits bacterial cell wall synthesis by means of binding to the penicillin-binding proteins, which in turn inhibition of the transpeptidation step in peptidoglycan synthesis which is required for bacterial cell walls [41].

Conclusion

In conclusion, Vancoplus demonstrated potent in vitro activity against Gram-positive staphylococcal, streptococcal isolates. The results of this surveillance study can serve as a benchmark for monitoring the in vitro activity of this new agent.

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Table 1: Distribution of isolates included in the study

Bacteria according to classification		Name of strains	Number of isolates
Staphylococci	Coagulase Positive	*S. aureus*	211
	Coagulase Positive	MRSA	130
	Coagulase Negative	*S. epidermidis*	15
Streptococci	α-hemolytic	*S. pneumoniae*	12
	β-hemolytic	*S. agalactiae*	9
	β-hemolytic	*S. pyogenes*	13
	γ- hemolytic	*S. bovis*	7
	γ- hemolytic	*E. faecalis*	11
		Total	408

Table 2: Comparative MIC values.

Name of micro-organisms	Total no of strains (408 clinical isolates)	Name of drugs											
		Vancoplus (Vancomycin+ceftriaxone)		Vancomycin		Linezolid		Daptomycin		Teicoplanin		Clindamycin	
	Values in µg/ml												
		S	R	S	R	S	R	S	R	S	R	S	R
S. aureus	211	0.0625-2	16-32	0.0625-2	16-512	1-4	8-64	0.0312-1	2-64	1-8	32-512	0.0625-0.5	8-128
MRSA	130	0.0625-2	16-64	0.0625-2	16-32	1-4	16-512	0.0312-1	8-64	1-8	2-64	0.0625-0.5	32-512
S. epidermidis	15	0.0625-2	16-256	0.0625-2	16-256	1-4	8-64	0.0312-1	2-64	1-8	32-512	0.0625-0.5	8-128
S. pneumoniae	12	0.0625-1	8-32	0.0625-1	8-256	1-4	8-64	0.0312-1	4-64	1-8	32-512	0.03125-0.25	0.5-64
S. pyogenes	13	0.0625-1	2-64	0.0625-1	2-128	1-4	32-128	0.0625-1	2-256	1-4	32-256	0.0625-0.25	2-128
S. bovis	7	0.0312-1	2-16	0.125-128	4-256	0.5-2	4-512	0.25-1	2-256	0.5-2	16-128	0.0625-0.25	2-128
S. agalactiae	9	0.0625-1	2-64	0.0625-1	2-128	1-4	32-128	0.0625-1	2-256	1-4	32-256	0.0625-0.25	2-128
E. faecalis	11	0.0625-4	32-128	0.0625-4	32-512	0.0625-2	8-512	1-4	8-64	0.0625-8	32-1024	0.0625-0.25	2-128
S. aureus ATCC43300	1	0.25		16		4		8		8		2	

S: Susceptible; I: Intermediate; R: Resistance.

Table 3: Antibiotic susceptibility pattern of gram positive organisms.

Name of micro-organisms	Total no of strains (408 clinical isolates)	Name of drugs											
		Vancoplus (Vancomycin+ceftriaxone)		Vancomycin		Linezolid		Daptomycin		Teicoplanin		Clindamycin	
	%												
		S	R	S	R	S	R	S	R	S	R	S	R
S. aureus	211	95.7	4.3	72.5	27.5	84.8	15.1	79.6	20.4	78.7	21.3	51.6	48.3
MRSA	130	90	10	26.1	73.8	70.7	29.2	26.1	73.8	43.8	56.1	19.2	80.7
S. epidermidis	15	93.3	6.6	40	60	60	40	46.6	53.3	53.3	46.6	33.3	66.6
S pneumoniae	12	91.6	8.3	41.5	58.3	66.7	33.3	58.3	41.6	66.6	33.3	25	75
S. pyogenes	13	100	-	46.1	53.8	84.6	15.4	61.5	38.5	61.5	38.5	23.1	76.9
S. bovis	7	100	-	57.1	42.8	71.4	28.6	57.1	42.8	71.4	28.6	42.8	57.1
S. agalactiae	9	100	-	88.8	11.1	77.8	22.2	55.5	44.4	66.6	33.3	44.4	55.5
E. faecalis	11	90.9	9.1	72.7	27.3	81.8	18.2	72.7	27.3	72.7	27.3	27.3	72.7
S. aureus ATCC43300	1	100	-	100		100			100		100		100

S: Susceptible; I: Intermediate; R: Resistance.