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Research Article

Surveillance Study for MRSA Prevalence and Susceptibility Trends Against *mecA* and *vanA* Positive Clinical Isolates

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is among a major cause of hospital acquired and community acquired infections. Present study demonstrates the prevalence of MRSA in various clinical samples collected from hospitals located in north and west India. This study also describes the prevalence of *mecA* and *vanA* genes and their resistance pattern among the drugs. In the current investigation, 313 clinical samples were collected over a period of one year from March 2013 to April 2014. Among these isolates, 210 isolates belonged to *Staphylococcus spp.* The highest prevalence for *Staphylococcus aureus* was observed in pus (96 %) closely followed by ear swabs (82.7 %) wound swabs (81.8%), blood (78%) and urine (68.2%). 56.2 % isolates were confirmed to be MRSA of which 36 % isolates carried *mecA* gene and 23 % isolates harboured *vanA* gene and 41 % isolate showed the presence of both *mecA* and *vanA* genes.

Susceptibility results advocated the superiority of novel antibiotic adjuvant entity Vancoplus over other tested comparative drugs with 87 % to 92 % susceptibility. Teicoplanin was the second most effective drug with susceptibility rates 46 to 75%. The susceptibility of other drugs, vancomycin, linezolid and daptomycin, was <40%, whereas none of the isolates was found to be susceptible against cefoperazone plus sulbactam and ceftriaxone. The results of the present study shed light on the increased resistance among MRSA isolates and prove the efficiency of Vancoplus as an effective alternative empiric therapy to the routinely prescribed drugs commonly used to treat MRSA infections.

Keywords: Antibiotic adjuvant therapy, Clinical isolates, Susceptibility, Vancoplus.

INTRODUCTION

Staphylococcus aureus is the most commonly isolated bacterial pathogen and is an important cause of skin and soft tissue infections (SSTIs), endovascular infections, pneumonia, septic arthritis, endocarditis, osteomyelitis, foreign-body infections and sepsis [1]. Untill the emergence of penicillin resistant pathogens in 1950s, penicillin (discovered in 1940s) was routinely used for the effective treatment of *S. aureus* infections [2]. Methicillin was first introduced in human medicine in 1960s for the treatment of infections caused by penicillin resistant *S. aureus* [3]. However, methicillin-resistant *Staphylococcus aureus* (MRSA) was first detected approximately 50 years ago and is still among the top three clinically important pathogens [4]. MRSA are prevalent worldwide and are considered as the most important cause of hospital-acquired infections (HAI) and community-acquired infections (CAI), resulting in increased morbidity and mortality in the hospital settings [5]. The widespread use of antimicrobial agents to treat staphylococcal infections has resulted in the emergence of resistant forms of these organisms.

The methicillin resistance in MRSA is due to the acquisition of *mecA* gene, which encodes the low-affinity penicillin-binding protein (PBP) 2a, a cell

wall transpeptidase, which, in conjunction with native PBP2, allows continued cell wall synthesis in the presence of β-lactams [6]. Apart from being resistant to methicillin, most MRSA have become resistant to number of other antimicrobial agents like semisynthetic penicillins (oxacillin, and nafcillin), macrolides, tetracycline, and aminoglycosides and thus has made the management of *staphylococcal* diseases a global challenge [7-9].

To overcome the multi drug resistance among *S. aureus* strains, vancomycin, a glycopeptide, was considered to be the best alternative for the treatment [10]. However, 30 years after its development, clinical isolates with reduced vancomycin susceptibility were described. The susceptibility of MRSA to vancomycin has been declining and reports of treatment failures are increasing [11-14]. For example, upto 40% failure rate in treating MRSA caused lower respiratory tract infections were reported [15]. Unfortunately, with the passage of time, use of vancomycin for *S. aureus* infections has been associated with an increased risk for recurrent bacteremia and mortality, which may be a due to inadequate bactericidal activity against *S. aureus* strains even with an MIC of 1-2 µg/ml [4,13-14]. Vancomycin resistance was first described in isolates of *Staphylococcus epidermidis* [16]. A varied level of vancomycin resistance was reported from different parts of the world [17-20]. Over a period of time, several different MRSA resistant genes emerged which fail to respond to standard of care.

Thus, there is a urgent need of alternative therapy to treat such MRSA cases. The aim of the present work was to find the prevalence of *S. aureus* and MRSA among various clinical samples and to do genetic characterization of these strains in order detect the *mecA* and *vanA* genes. Final objective was to evaluate the susceptibility of various drugs used commonly to treat these MRSA strains to find the best empiric therapy with high degree of susceptibility.

MATERIALS AND METHODS

Clinical isolates

A total of 313 clinical samples were collected over a period of one year from March 2013 to April 2014 from various hospitals of north and west India region. Of which, 210 *Staphylococcal* isolates consisting of 178 *S. aureus* were recovered. The clinical specimens consisting of pus (86), blood (61), urine (51), wound swabs (63) and ear swabs (52).

Media and culture conditions

All clinical samples except urine were first inoculated on to blood agar (Hi-Media, India) and MacConkey agar (Hi-Media, India) plates whereas the urine samples were inoculated only on cystine lactose electrolyte deficient (CLED) agar (Hi-Media, India) plates. The plates were incubated at 37°C for 24–48 h. The identification of isolates was done according to standard method described elsewhere [21].

Deoxyribonucleic acid (DNA) isolation and (polymerase chain reaction) PCR

DNA from each MRSA isolate as well as positive control was extracted using the method described earlier [2]. The PCR amplification of the *mecA* and *vanA* was done using the Eppendorf thermocycler (Germany). The primers and the PCR conditions were as described earlier [2]. For PCR amplifications, about 200 pg of DNA was added to 20 µL mixture containing 0.5 mM of dNTPs, 1.25 µM of each primer and 3.0 U of Taq polymerase (Merck Specialities Private limited, Mumbai, India) in 1x PCR buffer. The amplified products were separated in 1.5% agarose gel containing ethidium bromide. The gel images were taken under ultraviolet light using gel documentation system (Bio-Rad, USA). A 100 bp ladder molecular weight marker (Banglore genie) was used to measure the molecular weights of amplified products.

Minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination

MIC of each antibacterial agent was determined by agar dilution method using CLSI guidelines [22]. The MIC90 value represents the lowest dilution at which bacteria fail to grow at 37°C after 18 to 24 h of incubation. To determine MBC, 100 µL was aspirated from the wells where there was no visible growth of planktonic bacterial population in the MIC90 experiment and spread onto Mueller Hinton Broth agar (MHBA, Hi-Media, Mumbai, India) plates and incubated overnight at 37°C in incubator. MBC was read as the lowest antibiotic concentration to kill 99.9% of the initial inoculum.

Antibiotic susceptibility testing

Antimicrobial susceptibility testing was done by Kirby–Bauer disk diffusion method as recommended by the Clinical Laboratory Standards Institute [22] using discs of different antibiotics: vancomycin (30 µg), linezolid (30 µg), daptomycin (10 µg), ceftriaxone (30 µg), teicoplanin (30 µg), Vancoplus (30:15 µg) and cefoperazone plus sulbactam (75:30 µg). Inoculum of 0.5 McFarland standards turbidity was prepared in a Mueller Hinton Broth (MHB,

Mumbai, India) from isolated colony of pathogens selected from 18–24 hour agar plates. Within 15 minutes, a sterile cotton swab was dipped into the inoculum suspension. The swab was rotated several times and pressed firmly against the inside wall of the tube above the fluid level and inoculated on the dried surface of a MHA plate by streaking the swab over it. For even distribution of inoculum, the swab was streaked two more times at 60° over the agar surface. After 3–5 minutes, antibiotic discs were applied and pressed down to ensure complete contact with agar surface. The discs were distributed evenly to ensure a minimum distance of 24 mm from center to center. The plates are then inverted and incubated for 16-18 hrs aerobically at 37 °C within 15 minutes of disc application. The zone of inhibition around the wells was measured in mm (millimeter), averaged and the mean values were recorded

RESULTS

A total of 313 clinical samples were collected from various hospitals. Further characterization of these isolates yielded 210 (67.1 %) *Staphylococcus* species. Of these *Staphylococcus* species, 178 isolates of *S. aureus* were identified with highest prevalence in pus (96 %) closely followed by ear swabs (82.7 %), wound swabs (81.8 %) and blood (78 %). However, urine (68.2%) samples showed comparatively lower prevalence. All these *S. aureus* were coagulase positive. From a total of 178 *S. aureus*, 100 isolates found to be resistant to methicillin (Methicillin resistant S. *aureus* – MRSA), with highest observed prevalence in pus samples (73.2%) followed by urine (53.3 %), ear swab (50 %), blood (43.7 %) and wound swab (38.9%) (Table 1).

Detection of *mecA* and *vanA* genes in MRSA isolates

PCR results revealed that among 100 MRSA isolates, 36 (36 %) isolates showed the presence of *mecA* gene and 23 (23 %) isolates showed the presence of *vanA* gene. While 41 (41 %) isolate showed the presence of both *mecA* plus *vanA* genes.

MIC testing

The MIC values of the tested MRSA strains are depicted in Table 2. The MIC values of *mecA* positive isolates was the highest against cefoperazone+sulbactam (32-128 µg/ml) followed by ceftriaxone (16-64 µg/ml), vancomycin (2-32 µg/ml), daptomycin (4-16 µg/ml), teicoplanin (2-16 µg/ml), linezolid (2-8 µg/ml). However the MIC values of *mecA* positive isolates was least against Vancoplus ranging from 0.25-4 µg/ml. The MIC values of *vanA* positive isolates was the highest against cefoperazone+sulbactam (32-256 µg/ml), followed by ceftriaxone (32-128 µg/ml), vancomycin (2-32 µg/ml), both daptomycin and teicoplanin (4-16 µg/ml) and were low against linezolid (1-8 µg/ml). The least MIC values were observed against Vancoplus (0.25-4 µg/ml). For *mecA* and *vanA* positive isolates the highest MIC values observed for cefoperazone+sulbactam (128-512 µg/ml), followed by ceftriaxone (64-512 µg/ml), vancomycin (8-64 µg/ml), teicoplanin (4-32 µg/ml), daptomycin and (8-32 µg/ml) and were low against linezolid (2-16 µg/ml). Like in *mecA and VanA* positive isolates, the isolates with both *mecA* and *VanA* genes showed least MIC values in the range of 0.5-4 µg/ml for Vancoplus.

MBC testing

The MBC values for the tested MRSA isolates followed the same trend as that of the MIC values (Table 2). The MBC value for the cefoperazone + sulbactam combination was the highest (128->1024 µg/ml) against all the tested MRSA isolates (*mecA*, *VanA* and *mecA* + *vanA*). MBC values for *mecA* positive isolates against ceftriaxone was high (64-256 µg/ml) followed by vancomycin (16-128 µg/ml), daptomycin (32-128 µg/ml), linezolid (16-64 µg/ml), teicoplanin (8-64 µg/ml) and the least MBC was observed against vancoplus (1-32 µg/ml). For *VanA* positive, highest MBC value was observed against cefoperazone+sulbactam (128-1024 µg/ml) followed by ceftriaxone (128-512 µg/ml), vancomycin (32-256 µg/ml), daptomycin (32-128 µg/ml), teicoplanin (16-128 µg/ml), linezolid (8-64 µg/ml) and Vancoplus (1-32 µg/ml). And for both *mecA* and *vanA* positive isolates similar pattern of MBC values were observed with highest values observed for cefoperazone + sulbactam (1024->1024) and least for Vancoplus (2-32 µg/ml).

Antibiotic sensitivity testing

The antibiotic sensitivity study of the tested isolates showed variable sensitivity pattern with different antibiotics having different sensitivities. The most effective antibiotic was found to be Vancoplus with 87-92 % isolates being susceptible to it. Next to Vancoplus, teicoplanin was found to be second most effective drug with susceptibility rate 46 to 75%. The susceptibility of other drugs, vancomycin, linezolid and daptomycin, was <40% whereas none of the isolates was found to be susceptible against cefoperazone plus sulbactam and ceftriaxone (Figure 1 and 2).

DISCUSSION

S. aureus is innocuous in most environments with remarkable adaptability and versatility which has equipped it as a commensal and pathogen. It is one of the most infectious agent with high prevalence in various communities and healthcare institutions [23]. The present study showed high prevalence of *Staphylococcus species* (67.1%) in hospital acquired gram positive samples. Nwoire et al. [24] also reported high prevalence (60.4%) of *Staphylococcal* infection among hospital acquired samples. Among *Staphylococcus species*, infections caused by *S. aureus* and MRSA have been associated with high morbidity and mortality rates. In India, occurrence of MRSA infections varies from 30-80 % [25-28]. The prevalence of MRSA in clinical samples obtained from different hospitals was determined. Results of the present study showed 56.2 % of MRSA among the tested strains. Similar results were also reported in studies from north India with prevalence rates of 46 to 55 % [29-30]. Our results reflected highest MRSA prevalence in pus samples (73.2%), which is which is comparable to earlier study [31].

Resistance to methicillin is determined by the function of penicillin-binding protein 2 (PBP2, or PBP2a) that binds to β-lactam antibiotics with much lower affinity than the intrinsic set of PBPs of *S. aureus* [32-33]. In the present study 36 % isolates showed the presence of *mecA* gene. Pramodhini et al. [34] also reported 36.4% prevalence of *mecA* gene among MRSA isolates. Vancomycin has been considered the prime antimicrobial agent to treat serious infections caused by MRSA. However, in past few years, vancomycin intermediate and resistant *S. aureus* have been reported from many countries including India [35-42]. Dissemination of the *vanA* gene cluster from *E. faecalis* to *S. aureus* [43-44] has raised fears about the occurrence of such genetic transfer in clinical isolates of methicillin resistant *S. aureus*. In the present study 23 % isolates among MRSA isolates showed the presence of *VanA* genes, while 41% isolates showed the presence of both genes, advocating the probable transfer of *Van*A gene cluster to MRSA strains containing *mecA* gene.

Vancomycin-resistant *S. aureus* tend to be multidrug resistant against a large number of currently available antimicrobial agents, compromising treatment options and increasing the likelihood of inadequate antimicrobial therapy and increase in morbidity and mortality [45]. The MIC studies of the *mecA* and *VanA* gene positive isolates against seven different antibiotics demonstrated results which send alarming signals. In the present study, along with being resistant to vancomycin, *mecA* and *VanA* gene positive isolates showed resistance to a wide range of antimicrobial agents like ceftriaxone, daptomycin, teicoplanin, cefoperazone + sulbactam and linezolid. Salem-Bekhit et al. [46] also reported high resistance to both vancomycin and teicoplanin against *vanA* positive isolates. MBC values of the MRSA isolates against the tested antibiotics also followed the similar trend with highest MBC values observed against cefoperazone+sulbactam in all the (*mecA, VanA and mecA + VanA* positive) isolates. Antibiotic sensitivity profile showed considerable variability among the tested antibiotics. The isolates showed different levels of resistance to different antibacterial drugs. Linezolid resistance may be due to either spontaneous mutations or by a acquisition of a cfr (chloramphenicol-florfenicol resistance) gene. The cfr gene was initially described in a bovine Staphylococcus sciuri isolate [47-50]. The resistance towards daptomycin may be due to bacterial cell wall thickening [51] or due to the *S. aureus* strains carrying *mprF*, *yycG*, *rpoC* and *rpoB* mutant genes [52]. Resistance in S. aureus during treatment with daptomycin have been reported [53]. The observed daptomycin resistance in our study may be due to the prolonged usage of daptomycin to treat these infections. The inducible resistance to teicoplanin is observed in glycopeptide resistance strains having *vanA* genes [54]. A considerable teicoplanin resistance witnessed in our study may also be due to acquired resistance during treatment with teicoplanin. This worsened scenario with increased resistance among all the routinely used antibiotics is a prime concern now. However in contrast to these, all the MRSA isolates showed high level of sensitivity to Vancoplus, advocating the superiority of vancoplus in these MRSA strains which may be due to synergistic activity of components. Thus effectiveness of Vancoplus also signifies the importance of combination therapy over monotherapies especially when accompanied with adjuvants.

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Competing Interests

The authors have declared that no competing interests exist.

Table 1
Prevalence of MRSA in various clinical samples.

Clinical samples	Number of samples	Staphylococcus species (%)	*S. aureus* (%)	MRSA (%)
Pus	86	74 (86.0)	71 (96.0)	52 (73.2)
Blood	61	41 (67.2)	32 (78)	14 (43.7)
Urine	51	22 (43.1)	15 (68.2)	8 (53.3)
Wound swabs	63	44 (69.8)	36 (81.8)	14 (38.9)
Ear swabs	52	29 (55.8)	24 (82.7)	12 (50)
Total	313	210 (67.1)	178 (84.7)	100 (56.2)

Table 2
MIC and MBC values of Vancoplus and other competator drugs in *mecA*,*vanA* and *mecA*+*vanA* positive isolates.

Name of drugs	MIC (µg/ml)			MBC (µg/ml)		
	mecA	*vanA*	*mecA*+*vanA*	*mecA*	*vanA*	*mecA*+*vanA*
Vancoplus	0.25-4.0	0.25-4	0.5-4	1-32	1-32	2-32
Vancomycin	2-32	2-32	8-64	16-128	32-256	64-512
Ceftriaxone	16-64	32-128	64-512	64-256	128-512	256-1024
Linezolid	2-8	1-8	2-16	16-64	8-64	8-128
Daptomycin	4-16	4-16	8-32	32-128	32-128	64-512
Teicoplanin	2-16	4-16	4-32	8-64	16-128	32-512
Cefoperazone+ sulbactam	32-128	32-256	128-512	128-512	128-1024	512->1024

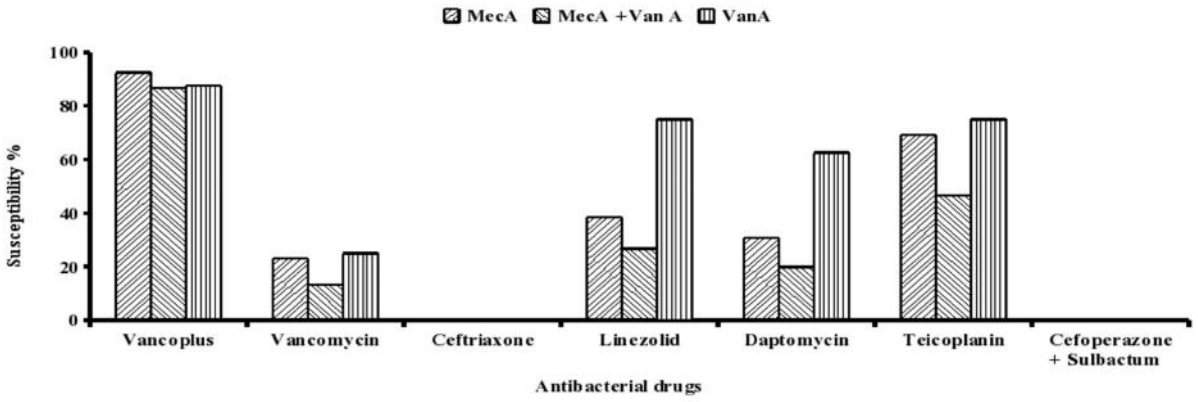

Figure 1
Susceptibility percentage of MRSA isolates towards different antibacterial drugs.

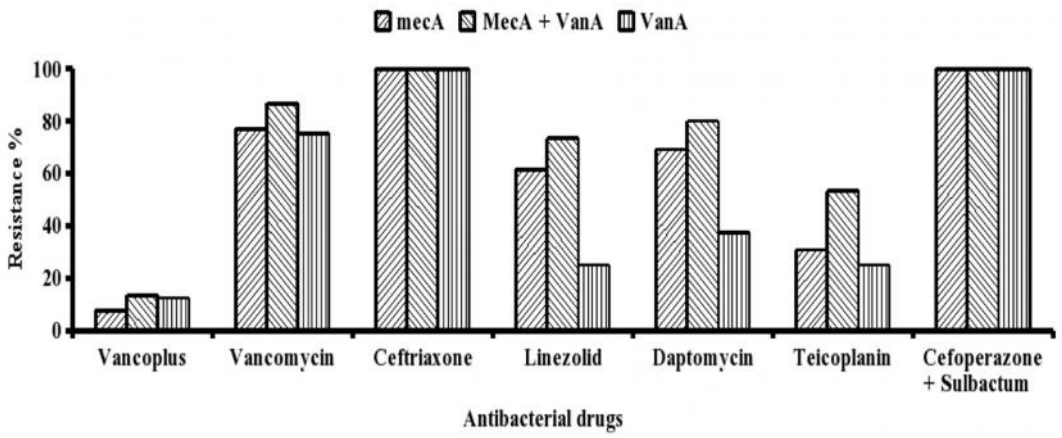

Figure 2
Resistance percentage of MRSA isolates towards different antibacterial drugs.

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