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OCCURRENCE OF VANCOMYCIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IN THE ORAL CAVITY OF PATIENTS WITH DENTAL CARIES

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Oral streptococci are the major group of microbes isolated from oral microflora. They represent frequent pathogens of infective endocarditis (IE), and it is assumed that in most of the cases oral streptococci are acquired via mucosa layer of oral cavity. *Staphylococcus aureus* is also frequently isolated from IE as it accounts for 20%–30% of all cases. Vancomycin has been the most reliable therapeutic agent against infections caused by methicillin-resistant *S. aureus* (MRSA). The main objective of this study was to examine the occurrence of *S. aureus* species in dental caries specimens. Antimicrobial susceptibility testing of *S. aureus* to four antibiotics namely vancomycin, linezolid, teicoplanin, and daptomycin was performed. Detection of vancomycin resistance was conducted using polymerase chain reaction. Among the tested 150 strains, 98 were MRSA and of that 54 were vancomycin sensitive and 27 were resistant. All 98 MRSA strains were positive for *mecA* and 36 yielded *pvl*, whereas 13 carried *vanA* and only 2 were positive for *vanB*. Majority of the isolates showed sensitivity toward daptomycin and linezolid. Strains of *S. aureus* exhibiting decreased susceptibility to different antibiotics like vancomycin, daptomycin, and linezolid severely compromise the therapeutic alternatives and require a considerable

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amount of time, public awareness, and integrative health-care strategies to prevent the emergence of resistance to these compounds.

Keywords: antibiotic resistance, multidrug-resistant bacteria, *S. aureus*, *vanA*, dental caries, *S. aureus*, antibiotics, vancomycin resistant

Introduction

Multidrug-resistant strains are arising worldwide. They have become a menace, which requires more attention and a driving force to eradicate them. Methicillin-resistant *Staphylococcus aureus* (MRSA) has been of increased concern and a major pathogen of hospital-acquired infections [1, 2]. Vancomycin was considered to be the best alternative for the treatment of multidrug-resistant MRSA. However, there have been increasing reports of vancomycin-resistant *S. aureus* (VRSA) strains exhibiting two different resistance mechanisms [3, 4]. Over the past two decades, vancomycin has been the drug of choice for MRSA, but recent studies have indicated the therapeutic failure of vancomycin against MRSA isolates. It has aroused global concern since only a limited number of effective therapeutics are currently available against MRSA [5, 6]. There have been reports of MRSA encoding both *mecA* and *mecC* confer resistance to β -lactams and poses diagnostic problems [7]. In various studies, it has been noted that MRSA strains were also resistant to one or more non- β -lactam antibiotics and some of them were multidrug-resistant [8]. Oral streptococci are the major group of microbes isolated from oral microflora and they are frequently isolated pathogens from infective endocarditis (IE). One of the clinical importance of dental caries is the inimitable site for these bacteria to enter bloodstream and increases chances to infect heart. Therefore, dental procedures and dental hygiene have been given much more attention to the predisposing conditions and to prevent streptococcal IE [9].

Besides streptococci, reports suggest a high incidence of *Staphylococcus* species in IE. Among them, *S. aureus* is the most frequently isolated bacterium, which accounts for 20%–30% of all IE cases [10, 11]. Though influencing conditions and route of infections related to staphylococcal IE are not indicated. However, in most of cases, it is assumed that it is acquired via a mucosal surface or percutaneous route, particularly in cases associated with nosocomial infection [12].

Staphylococci are main inhabitant of skin, as well as glands of skin. These are also found to be briefly resident in the oral cavity and it is speculated that IE cases caused by them are originated in the oral cavity [13].

In this study, we examined the occurrence of *S. aureus* in dental caries specimens. In addition, in view of the abovementioned circumstances, sensitivity

patterns of *S. aureus* to four different antibiotics – vancomycin, linezolid, teicoplanin, and daptomycin were analyzed. The prevalence of vancomycin resistance was detected using polymerase chain reaction (PCR).

Materials and Methods

Bacterial isolates

A total of 150 *S. aureus* isolates were obtained from dental caries clinical samples from the patients who were visited the facilities of dental hospitals clinic. The morphology and characteristics of *S. aureus* strains were studied using Gram staining, hemolysis on blood agar, DNase, catalase, coagulase tests, and fermentation of mannitol. The study was designed and permitted by the Institutional Ethics Committee and a written informed consent was obtained from every individual before the sample collection.

Phenotypic methods for detection of MRSA

All the isolates were subjected to disk diffusion susceptibility testing to oxacillin (1 µg) and ceftioxin (30 µg) disks for detection of methicillin resistance. Growth on oxacillin screen agar containing 6 µg/ml of oxacillin was also checked on all the isolates. MRSA (ATCC 43300) was used as positive control and *S. aureus* (ATCC 25923) as negative control. MRSA strains were further tested.

Determination of minimum inhibitory concentration (MIC)

MIC was determined based on Clinical and Laboratory Standards Institute guidelines. Briefly, gradient plates of Mueller–Hinton agar (Hi Media Laboratories, Mumbai, India) were prepared with vancomycin, daptomycin, teicoplanin, and linezolid (0.5–256 µg/ml, Sigma-Aldrich). An amount of 0.5 McFarland equivalent inoculum prepared using 18- to 24-h-old culture was spotted on to gradient plates and the plates were incubated overnight at 35 °C for 24 h to achieve visible growth.

PCR amplification for all vancomycin-resistant genes

A single target PCR was carried out to detect the presence of genes encoding vancomycin resistance. Of the many genotypes of vancomycin resistance described in enterococci, attempt was made to identify the commonest ones, PCR

amplification of the *vanA* gene was performed using GeneAmp thermal cycler (Applied Biosystems, Wilmington, NC, USA).

PCR for MRSA detection

mecA gene encoding methicillin resistance and *pvl* gene presented in community-associated MRSA strains were tested in all the 98 MRSA isolates using the primers given in Table I.

The amplification conditions maintained were initial denaturation at 98 °C for 10 s, annealing for 1 min at different temperature respective to target PCR, polymerization was performed at 72 °C for 1 min, and finally extension at 72 °C for 5 min.

Results and Discussion

During the study period, a total number of 150 isolates were collected from dental caries clinical samples. Altogether 98 were found to be MRSA by disk diffusion test. Among the 98 patients, 59 of them had prolonged hospital stay of longer than 10-day duration, 42 patients had postoperative dental infections, and 29 patients were admitted due to dental infections for more than 2 days. In all, 71 patients were males and 27 patients were females.

The results of MIC for 98 isolates showed vancomycin was <2 µg/ml in case of 54 isolates and 27 isolates showed intermediate resistance in the range of 4–8 µg/ml, whereas 15 isolates showed MIC in the range of 16–128 µg/ml indicating that these were VRSA. Detailed results are shown in Table II.

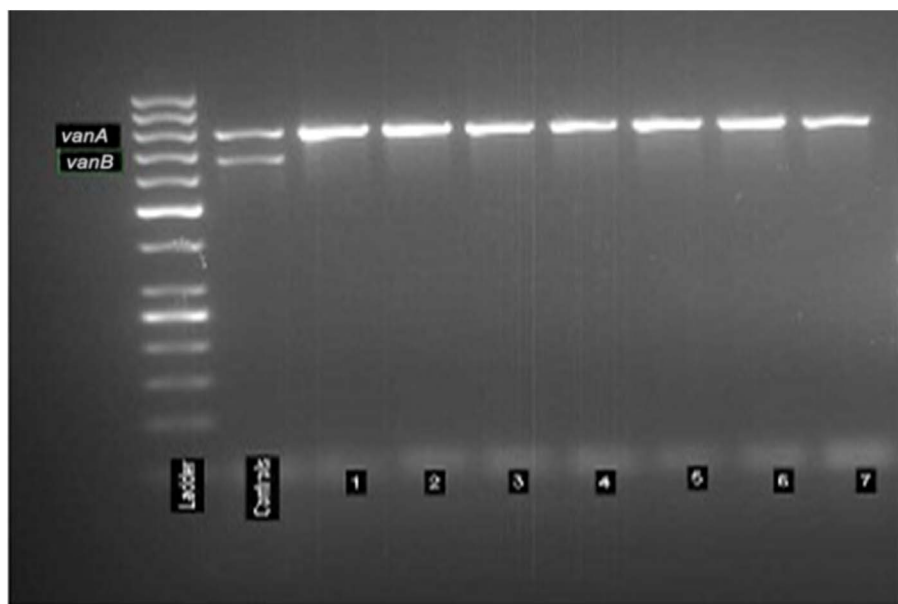
By PCR amplification, *mecA* gene was present in all the 98 isolates of MRSA and *pvl* gene was present in 36 isolates. *vanA* gene was present in 13 isolates, and only 2 were *vanB*-positive (Figure 1).

Table I. List of primers used in this study

Gene	Forward	Reverse	Reference
<i>vanA</i>	AATAGCGCGGACGAATTGGAC	AACGCGGCACTGTTTCCCAA	[14]
<i>vanB</i>	CTTAACGCTGCGATAGAAGC	CTGATGGATGCGGAAGATAC	[14]
<i>vanD</i>	TTTGTAAGCCTGCCCGTTC	CCAAGTAYCCGGTAAATCTTC	[14]
<i>vanE</i>	AAATAATGCTCCATCAATTTGCTGA	ATAGTCGAAAAAGCCATCCACAAG	[14]
<i>vanG</i>	TTGGAGGCAATTCAACAGAGT	TCGCAGCCAACAACAGGTATT	[14]
<i>mecA</i>	GTAGAAATGACTGAACGTCCGATAA	CCAATTCCACATTGTTCCGGTCTAA	[15]
<i>pvl</i>	ATCATTAGTAAAATGTCTGGACATGA	GCATCAASTGTATTGGATAGCAAAAAGC	[15]

Table II. List of positive isolates with their antibiogram and PCR results

Isolates	Vancomycin-resistant gene	Resistance	Susceptibility
VRSA1	<i>vanB</i>	Vancomycin	Daptomycin, linezolid, and teicoplanin
VRSA2	<i>vanA</i>	Daptomycin, vancomycin, and teicoplanin	Linezolid
VRSA3	<i>vanA</i>	Daptomycin, vancomycin, and teicoplanin	Linezolid
VRSA4	<i>vanA</i>	Vancomycin and teicoplanin	Linezolid and daptomycin
VRSA5	<i>vanA</i>	Vancomycin, daptomycin, and teicoplanin	Linezolid
VRSA6	<i>vanB</i>	Vancomycin	Linezolid, daptomycin, and teicoplanin
VRSA7	<i>vanA</i>	Vancomycin and teicoplanin	Linezolid and daptomycin
VRSA8	<i>vanA</i>	Vancomycin and teicoplanin	Linezolid and daptomycin
VRSA9	<i>vanA</i>	Vancomycin and teicoplanin	Linezolid and daptomycin
VRSA10	<i>vanA</i>	Vancomycin and teicoplanin	Linezolid and daptomycin
VRSA11	<i>vanA</i>	Vancomycin and teicoplanin	Linezolid and daptomycin
VRSA12	<i>vanA</i>	Vancomycin, daptomycin, and teicoplanin	Linezolid
VRSA13	<i>vanA</i>	Vancomycin, daptomycin, and teicoplanin	Linezolid
VRSA14	<i>vanA</i>	Vancomycin, daptomycin, and teicoplanin	Linezolid
VRSA15	<i>vanA</i>	Vancomycin, daptomycin, and teicoplanin	Linezolid

**Figure 1.** PCR amplification of *vanA* and *vanB* genes

For remaining antibiotics, the range of MIC was <1 g/ml for daptomycin, <8 g/ml for teicoplanin, and <4 g/ml for linezolid and it was found that majority of the isolates showed sensitivity toward daptomycin and linezolid antimicrobials and only few were resistant isolates. The isolates, which were *vanA*-positive, have been illustrated in Table I. All these isolates were found to be highly resistant to all the antibiotics, which were tested in this study.

The rising risk of MRSA strains has proved to be a great problem globally and a positive and healthy approach has to be taken to combat this disease scourge. Vancomycin has been the most reliable therapeutic agent against infections caused by MRSA. A certain group of *S. aureus*, designated hetero-VRSA, frequently generate VRSA upon exposure to vancomycin, and are associated with infections that are potentially refractory to vancomycin therapy [16]. Although *vanA*-positive isolates have been found earlier in enterococci, but presence of *vanA* gene in *S. aureus* poses a great challenge, and it is considered to be due to conjugative transfer of the gene from enterococci to *S. aureus* during hospital-acquired infections [17].

S. aureus is the most commonly isolated *Staphylococcus* species in IE cases [18, 19], *Staphylococcus epidermidis* and *Staphylococcus lugdunensis* are also predominant causative agents of IE [11, 19], and aggressive clinical courses with high mortality [20]. Both *S. aureus* and *S. epidermidis* are found in patients with dental infection (particularly abscess); however, the possible presence of *S. aureus* is especially important in dental care due to its increased resistance. Earlier literature states that *S. aureus* amplified significantly after surgical induction of hyposalivation [14].

Among collected 150 isolates, 98 (65.3%) were MRSA and 42 (28%) were resistant to vancomycin. All 98 MRSA isolates were positive for *mecA* gene and only 13 (8.6%) and 2 (2.13%) harbored *vanA* and *vanB*, respectively. About 13 patients had *vanA*-positive strains, which indicate that recommended infection control strategies worked and prevented further spread to other patients, but still the exact mechanism of pathogenesis remains unknown. Most of the VRSA strains were also oxacillin or methicillin resistant (MRSA).

Conclusions

Strains of *S. aureus* exhibiting decreased susceptibility or resistance to vancomycin, daptomycin, and linezolid severely compromise the therapeutic alternatives for life-threatening MRSA infections. Strategies to prevent emergence of resistance to these compounds in MRSA appear to be tough: first, an adequate decrease in the bacterial load by timely surgical drainage of

collections and second, optimization of the pharmacological parameters of the antibiotics in specific clinical situations. An additional strategy could be a combination of treatments used against the widespread evolution of MRSA strains. Vancomycin resistance is thought to be due to increased overuse of the antibiotic in cases such as in pseudomembranous colitis or it may be due to certain changes in the bacterial cell wall structure such as thickening of the cell wall or changes in the site of action of the glycopeptides [15]. Preventing the scourge of multidrug-resistant strains particularly MRSA and VRSA will require a considerable amount of time, public awareness, and an integrative health-care approach.

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Conflict of Interest

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the article.

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