

Full Length Research Paper

Characterization of *Staphylococcus aureus* isolated from human dental infection

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***Staphylococcus aureus* is an opportunistic pathogen causing dental infection and systemic infections in human body. This organism decreases susceptibility to several types of antibiotics every day and becomes more resistant which is a growing sense of concern in this era. Considering this fact, the study was attempted to characterize the *S. aureus* from human dental infection and to determine the antibiogram profile of isolates. Sixty four (64) samples were collected from the patients with dental infection who visited different dental clinics and hospitals in Mymensingh, Bangladesh for treatment. Isolation and identification of *S. aureus* were conducted by using cultural, morphological, and biochemical characteristics. Polymerase chain reaction was performed for final confirmation of *S. aureus* followed by detection of methicillin resistant *S. aureus* (MRSA) targeting *mecA* and *mecC* genes. Antibiotic susceptibility test of isolated bacteria was tested against seven antibiotics by disk diffusion methods. Forty isolates among 64 samples were found positive for *S. aureus* based on cultural characteristics. Among them 30 isolates were found positive in coagulase test. Depending on the result of coagulase test, all the 30 isolates were subjected to antibiotic sensitivity test and among them 25 were 100% resistant to penicillin, ampicillin and amoxicillin. All the 25 isolates were subjected to polymerase chain reaction (PCR) to identify methicillin resistant gene *mecA* and *mecC*. Eight isolates were positive for *mecA* gene while no isolates were positive for *mecC*. The present findings conclude that *S. aureus* is prevalent in dental infections and contain methicillin resistant genes.**

Key words: Dental infection, *Staphylococcus aureus*, antibiotic resistance, methicillin resistant *S. aureus* (MRSA).

INTRODUCTION

Human oral cavity acts as a growth medium for pathogenic microorganisms due to its moistures, temperature and nutrient content such as lipid, carbohydrate and protein (Mohapatra et al., 2012). There

are several types of dental infections which occur in a patient's oral cavity such as tooth decay, periodontal disease, dental ache, dental plaque, dentin hypersensitivity, dental abscess, dental calculus,

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hyperdontia, malocclusion, acid erosion, acute necrotizing ulcerative gingivitis, dental fluorosis, tooth impaction, etc. *Staphylococcus aureus* is a putative pathogen of many oral diseases, such as oral mucositis, periodontitis, peri-implantitis, endodontic infections and even dental caries (Gibson et al., 2000; Heitz-Mayfield and Lang, 2010; Poeschl et al., 2011; Passariello et al., 2012).

S. aureus is a Gram-positive, non-motile, non-spore forming grape like clusters and the most important coagulase positive pathogen from staphylococci due to combination of toxic mediated virulence, invasiveness and antibiotic resistance (Loir et al., 2003). Some strains of *S. aureus* have developed drug resistance (Faden, 2018). Methicillin-resistant *S. aureus* (MRSA) (Rajadurai et al., 2006) is the strains of *S. aureus* that obtained resistance to beta-lactam antibiotics, which incorporates such as penicillin, amoxicillin, ampicillin, methicillin, oxacillin, cephalosporins, etc. (David and Daum, 2010). The propensity of *S. aureus* to acquire antibiotic resistance has prompted worldwide dissemination of clone expressing various antimicrobial resistances. Several hospital and nonhospital bacterial diseases are caused due to MRSA strains and sometimes lead to death (Bannerman and Peacock, 2007; Moussa et al., 2011; Peters et al., 2013; Faden, 2018).

Contaminations due to *S. aureus*, including the MRSA strains has long been common in Bangladesh. Because, indiscriminate use of antibiotics being a typical practice, hospital environments are not sufficiently clean as well as congestion of patients and attendants support spread of the infectious agents including *S. aureus* (Khan et al., 2007). The possible presence of *S. aureus* is particularly important in dental caries because of its increased resistance (Vellappally et al., 2017). So it is very logical to check the status of microbial resistance against commonly used antibiotics for the treatment of dental infections occurred by *S. aureus*. Considering the fact that the present study aimed to characterize *S. aureus* in human dental infections collected from the patients of different clinics and hospital of Mymensingh district, Bangladesh.

MATERIALS AND METHODS

Sample collection

A total of 64 samples of human dental infection were collected from different private clinics and Mymensingh Medical College Hospital (MMCH), Mymensingh during the period from January to March, 2018. The samples were collected directly by rubbing the infected teeth with sterile cotton buds and immediately placed them into nutrient broth and transferred to the laboratory within 1 to 2 h.

Isolation and identification of bacteria

Each nutrient broth containing samples were incubated at 37°C for

4 h for enrichment. Samples were then streaked on *Staphylococcus* species specific growth media mannitol salt agar (HiMedia, India) and incubated at 37°C for 48 h to get single colonies. The suspected colonies were further identified as *S. aureus* after culturing onto mannitol salt agar (MSA) and 5% sheep blood agar. Gram's staining and biochemical test including coagulase and catalase tests were performed for further confirmation of *S. aureus* (Cheesbrough, 1985).

Antimicrobial susceptibility test

Antibiotic susceptibility test was conducted using disk diffusion method or Kirby-Bauer (Bauer et al., 1966). All the coagulase positive isolates of *S. aureus* were subjected to antibiotic sensitive test against seven antibiotics namely penicillin (10 µg), ampicillin (20 µg), amoxicillin (30 µg), ciprofloxacin (5 µg), tetracycline (30 µg), erythromycin (15 µg) and cephradine (30 µg) (HiMedia, India) which were generally prescribed by physicians in the study areas. The zone of inhibition produced by *S. aureus* against each antibiotic was measured and interpreted as resistant, intermediate resistant and susceptible according to standards of Clinical Laboratory and Standards Institute (CLSI, 2016).

Molecular characterization of *S. aureus*

For final confirmation, all the coagulase positive isolates of *S. aureus* were subjected to molecular test as methods described by Stuhlmeier and Stuhlmeier (2003). Boiling method was followed for genomic DNA extraction (Dashti et al., 2009). Furthermore, penicillin and penicillin like (ampicillin and amoxicillin) antibiotic resistant isolates of *S. aureus* were subjected polymerase chain reaction (PCR) for detection of *mecA* and *mecC* genes using previously published primers (Lee, 2003; Stegger et al., 2012). The list of primers used in the study is shown in Table 1.

RESULTS AND DISCUSSION

Out of 64 samples of dental infection, 40 (62.5%) samples were found positive for *S. aureus* by observing cultural and morphological characteristics (Table 2). *S. aureus* growth in MSA appeared as golden yellow pigmented colonies and Gram's staining exhibited Gram positive, cocci shaped and arranged in grape like cluster under microscope. These findings are similar to the findings of Ray and Ryan (2003). The percentage of *S. aureus* isolated from human dental infection is more frequent as the findings of Ohara-Nemoto et al. (2008) reported 46.4%. McCormack et al. (2015) and Kim and Lee (2015) reported 18 and 36.6% *S. aureus* in human oral and perioral infections that is comparatively lower than the current results.

All the 40 isolates which were assumed positive for *S. aureus* were subjected to catalase and coagulase test where all of the isolates found positive for catalase test and 30 of them for coagulase test. Bubble formation in catalase and curd like clot formation in coagulase test indicated that the samples were positive for *S. aureus* indicating their ability to breakdown the hydrogen-peroxide to release free oxygen and plasma coagulation by activation of prothrombin (Karmakar et al., 2016). Of

Table 1. Primers with nucleotide sequences.

Primer	Sequence	Size (bp)	Reference
<i>Staphylococcus aureus</i>	5'-GGAGGAAGGTGGGGATGACG-3'	241	Stuhlmeier and Stuhlmeier (2003)
<i>Staphylococcus aureus</i>	5'-ATGGTGTGACGGGCCGGTGTG-3'		
<i>mec A</i> (F)	5'-AAAATCGATGGTAAAGGTTGG-3'	533	Lee (2003)
<i>mec A</i> (R)	5-AGTTCTGGCACTACCGGATTTTGC-3'		
<i>cC</i> (F)	5'-GAAAAAAGGCTTAGAACGCCTC-3'	138	Stegger et al. (2012)
<i>mecC</i> (R)	5'- GAAGATCTTTCCGTTTTTCAGC- 3'		

Table 2. Cultural and biochemical characterization of *S. aureus*.

Total no. of sample	No. of <i>S. aureus</i> positive isolates	No. of catalase positive isolates	No. of coagulase positive isolates	No. of isolates showing hemolysis on blood agar
64	40	40	30	30

Table 3. Molecular characterization of *S. aureus*.

No. of total sample	No. of coagulase positive sample (%)	No. isolates positive for <i>S. aureus</i> in PCR (%)	No. isolates resistant to penicillin, ampicillin and amoxicillin in antibiotic sensitivity test (%)	No. of isolates used for PCR targeting <i>mecA</i> and <i>mecC</i> gene (%)	No. of positive isolates in PCR	
					<i>mecA</i> gene	<i>mecC</i> gene
64	30 (46.87)	30 (46.87)	25 (39.06)	25 (39.06)	8 (12.5)	0 (0)

the 40 isolates of *S. aureus*, 30 of them created β -hemolytic character in blood agar. The findings of the present study were higher than the findings of Karmakar et al. (2016) who reported 40% of β -hemolysis in blood agar.

Dental patients usually take antibiotics primarily to treat postoperative and secondary infections. All 30 coagulase positive isolates of *S. aureus* were subjected to antibiotic susceptibility test and the results revealed that 93.33% isolates were found resistant to penicillin followed by 90% to amoxicillin, 83.33% to ampicillin and 33.33% to erythromycin where 80% sensitive to cephadrine, 63.33% to ciprofloxacin, and 60% to erythromycin (53.33%) (Table 3). Kim and Lee (2015) reported that *S. aureus* isolated from the periodontal patients showed 88% (36/41) isolates resistant to penicillin and ampicillin which is comparable to the present study. They also observed the susceptibility rate and found 98% isolates susceptible to cefotetan, 95% to ciprofloxacin and 90% to tetracycline and erythromycin that is comparatively higher than the current study. Similar antimicrobial susceptibility results were reported by previous authors (Rajadurai et al., 2006; Khan et al., 2007; Kim, 2012; Naeem et al., 2012). The higher resistant rate to commonly used antibiotics indicates indiscriminate or haphazard use that may have effect on treatment cost, poor prognosis as well as enhance the bacterial infection and growth virulent

pathogens.

Coagulase positive isolates of *S. aureus* were finally confirmed by molecular test using polymerase chain reaction (PCR) and all them were found to be positive (Figure 1) which showed the relevance of the statement of Ohara-Nemoto et al. (2008). A total of 25 isolates found resistant to penicillin, ampicillin and amoxicillin which were further conducted to molecular characterization to detect *mecA* and *mecC* genes (Table 4). Out of 25 isolates, 8 were found positive for *mecA* and no isolates found *mecC* positive (Figures 2 and 3). It is a new gene which is emerging in the world especially in European countries and spreading among human and animals throughout the world which follows the statement of Paterson et al. (2014) but not for Bangladesh. The prevalence of *mecA* gene was 12.5% which was higher than the findings of Ayepola et al. (2015) who reported 2.4%. Smith et al. (2003) reported 6% MRSA positive isolates in oral infection. Another study conducted by Renvert et al. (2008) in Sweden observed similar finding connected to patients of periodontal infections. According to Kurita et al. (2006), dental patients are not only responsible for spreading MRSA, health professional may transmit this pathogen through their instruments. There are on established guideline for controlling MRSA but Centers for Disease Control and Prevention (CDC) recommends some standard precautionary measure that

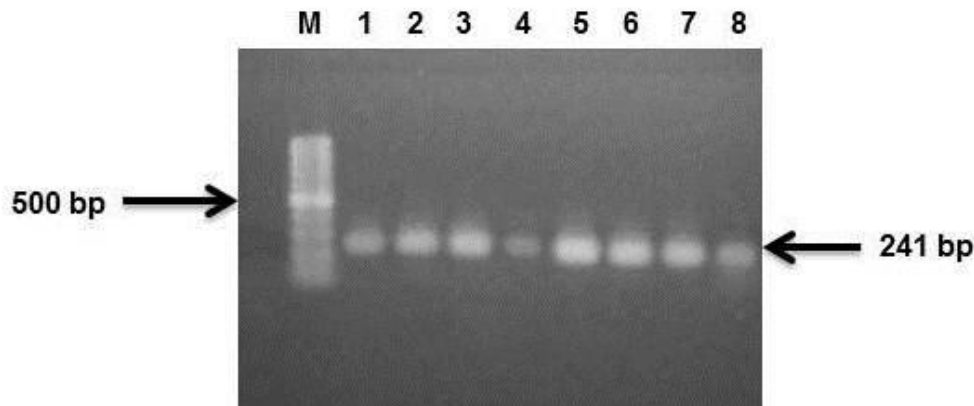


Figure 1. PCR for amplification of *S. aureus*. Lane M: DNA marker, Lane 1-7: tested isolates, lane 8: positive control.

Table 4. Antimicrobial susceptibility test.

Antimicrobial agent	Group	No. of isolates (%)		
		R	IR	S
Penicillin	Beta-lactam	28 (93.33)	2 (6.67)	0 (0)
Ampicillin	Beta-lactam	25 (83.33)	5 (16.67)	0 (0)
Amoxicillin	Beta-lactam	27 (90)	3 (10)	0 (0)
Ciprofloxacin	Quinolone	5 (16.67)	6 (20)	19 (63.33)
Tetracycline	Tetracycline	8 (26.67)	4 (13.33)	18 (60)
Erythromycin	Macrolide	10 (33.33)	4 (13.33)	16 (53.33)
Cephadrine	Cephalosporin	4 (13.33)	2 (6.67)	24 (80)

R- Resistant; IR- Intermediate resistant; S- Sensitive.

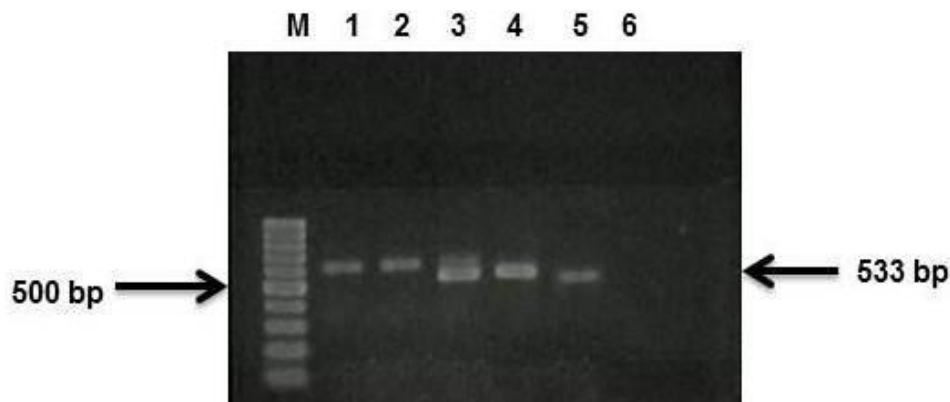


Figure 2. PCR for amplification of *mecA* gene of *S. aureus*. Lane M: DNA marker, Lane 1-4: tested isolates, lane 5: positive control, lane 6: negative control.

might help to reduce the spread of MRSA in dental patients (Harte, 2010). The present results revealed that *mecA* positive but not *mecC* positive isolates of *S. aureus* are associated with dental infection in Mymensingh city of Bangladesh.

Conclusion

Prevalence of *S. aureus* in dental patients is quite high and showed resistance to commonly used antibiotics as well as carried MRSA. Despite these results, the sample

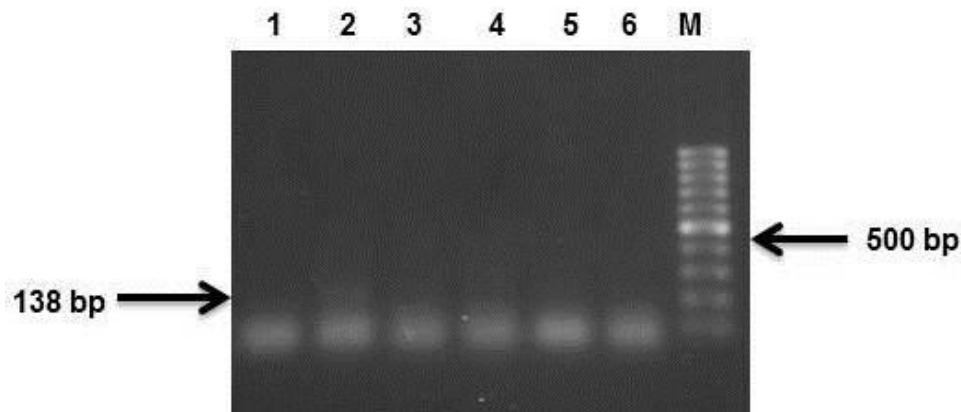


Figure 3. PCR for amplification of *mecC* gene of *S. aureus*, Lane M: DNA marker, Lane 1-6: tested isolates.

size of this study is not sufficient and study period was too short to uncover actual picture of MRSA involved in dental infection in Mymensingh, Bangladesh. Large scale studies could be done both in hospitalized patients and in community to identify prevalence of MRSA, genome analysis, identification of toxin gene and other antibiotic resistant gene. Regular brushing of teeth, keeping up oral cleanliness and consulting with dental doctors to check up the teeth once in a month should be taken to maintain a distance from dental infections.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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