Isolation and Characterisation of Bacterial Species from Patients with Dental Caries and Caries-free Subjects

ABSTRACT

Background  The oral cavity harbours a large number of bacterial species as normal flora existing as biofilm. Dental disease such as dental caries results when there is a shift in the balance of bacteria towards pathogenic species within these biofilms.

Objective  The objective of this study was to isolation, identification and characterisation of oral bacterial species of patients with dental caries and caries-free healthy control subjects.

Materials and Methods  A standard bacteriological procedures were followed in the isolation of bacteria. The identification of bacteria was carried out using matrix-associated laser desorption ionisation–time of flight–mass spectrometry (MALDI–TOF–MS) (Bruker MALDI Biotyper system). The characterisation of bacteria involved in the determination of biofilm forming potential and assessment of synergistic antimicrobial action of manuka honey and gentamicin against the oral species.

Results  A total of 13 bacterial species were isolated from 35 oral samples (10 from patients with dental caries); of which seven bacterial species have been isolated for the first time in Saudi Arabia. The Streptococcus spp. exhibited varied biofilm-forming potential and response to synergistic antimicrobial activity of manuka honey and gentamicin.

Conclusion  The isolation of seven bacterial species for the first time from dental caries and caries-free subjects in Saudi Arabia warrants a larger prevalence study involving molecular and phenotypic tests to assess their role in health and disease in Saudi population.

KEYWORDS  dental caries, biofilm, oral microbiology, streptococcus, gentamicin, MALDI–TOF–MS

INTRODUCTION

Dental caries and periodontal diseases are the major infectious diseases on a global scale. In addition to causing oral diseases, oral microorganisms are responsible for a wide range of systemic diseases beyond oral cavity. Oral microorganisms primarily live as dental plaque; which are complex polymicrobial biofilms. Oral biofilms are functionally and structurally organised polymicrobial communities embedded in self-produced extracellular matrix (EM), consisting mainly of polysaccharides, proteins and nucleic acids on mucosal and dental surfaces. Because of its contact with the external environment, the population structure of the bacterial flora in the mouth is a dynamic one with changes occurring at infinite rates. Nevertheless, relatively established bacterial biofilm communities are found on the buccal mucosa, tooth surfaces, gingival crevices, in the mucosal surfaces of the tongue and prostheses.

In healthy individuals, commensal bacterial form biofilms and provide benefit to the host. Oral disease is associated with a shift in the balance of bacteria towards pathogenic species within these biofilms. Cells growing in biofilm often exhibit altered phenotypes, such as increased resistance (10–1000 fold) to antibiotics (penetration of EM by drug is hindered) and decreased clearance by the immune system. These biofilm-specific phenotypes confer the pathogens with increased potential to cause disease, and according to an estimate by the National Institute of Health (NIH), USA more than 60% of all bacterial infections are caused by biofilm bacteria.
Honey is one of the oldest traditional medicines, which is being used in the treatment of several human ailments since the origin of mankind. Substantial efforts are being made to formulate effective therapy against bacterial infections by combining antimicrobial agents with different mode of action, as this approach reduces the likelihood of development of resistance to both agents simultaneously. Studies have shown that honey acts synergistically with different antibiotics against a variety of pathogens including Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae and Acinetobacter baumannii.

In this study, we isolated and characterised oral bacteria from patients with dental caries and healthy subjects and determined their potential to form biofilm. In addition, we also determined the synergistic antimicrobial action of honey and gentamicin against these oral species.

**MATERIALS AND METHODS**

**Isolation of bacterial species**

*Isolation from caries-free healthy controls:* Oral swab samples were taken from healthy mouth of the medical students at the College of Medicine, University of Hail. Sterile swab sticks were rubbed against the teeth three times to collect samples. Exclusion criteria were visit to dentist in last 3 months and use of antibiotic in last 1 month. Samples were plated on blood agar plates and chocolate agar plates and incubated at 37°C for 24 h.

*Isolation of bacterial samples from patients with dental caries:* The samples were taken from patients attending the dental section of Salamat Clinic, Hail, Saudi Arabia, for the treatment of dental caries. The samples were collected either by resident dentist or the staff nurse. They were plated on blood agar plates and chocolate agar plates and incubated at 37°C for 24 h.

**Identification of bacterial species**

Identification of bacteria was carried out using matrix-associated laser desorption ionisation–time of flight–mass spectrometry (MALDI–TOF–MS) (Bruker MALDI Biotyper system) according to the manufacturer’s guidelines. A single colony of a subculture was directly deposited in duplicate on a MALDI–TOF–MS plate (Bruker Daltonik GmbH, Germany), and the results were noted as described earlier.

**Production of biofilm**

Crystal violet dye-binding procedure was used to determine biofilm production potential of the bacterial isolates as described earlier. Bacterial strains were grown overnight on blood agar plates and a loop full of bacteria was suspended in brain heart infusion broth (BHIB) to ~10^6 cells per ml. Ten microliter of the cell suspension was added to the wells of flat-bottomed microtitre plates containing 200 µl of BHIB. The plates were then incubated overnight at 37°C for 24 h for biofilm formation. The spent media along with free floating, planktonic bacteria were discarded, and the wells of the microtitre plates were washed (2×) with normal saline to remove the unbound bacteria. Biofilms attached to the wells of the plates were then stained with 0.1% (w/v) crystal violet solution for 10 min at room temperature. The plate were washed (2×) to remove unbound dye, and the plates were observed visually for biofilm. (Fig 1A and Fig 1B).

**Synergistic antibacterial action of manuka honey and gentamicin**

To screen for antibiotic and manuka honey combinations with potential synergistic activity, disc diffusion tests were carried out using nutrient agar (NA) plates. Test bacteria were spread on NA plates and two gentamicin disks and one filter paper disk (sterile) were placed at equal distance from each other in a triangular fashion. Approximately 20 µl of honey was added to the filter paper disk and one of the gentamicin disks, which served as the synergistic activity-testing disk. The plates were then incubated at 37°C for 24 h, and the zones of inhibition were recorded.

**RESULTS AND DISCUSSION**

Despite advancements in oral disease science, dental caries continues to be a worldwide health concern, affecting humans of all ages, especially children with oral infection is on the rise. Dental caries is very common and up to 80% of the world population may suffer from some form of this disease during their lifetime. Culture-independent metagenomic studies revealed that more than 6 billion bacteria are present in the oral cavity of human representing more than 700 bacterial species which contribute to the health and physiological status of the oral cavity; only a fraction of which can be cultivated in conventional laboratory media.

*Fig. 1* Biofilm production by strong biofilm producer Streptococcus salivarius (graded 4+; Fig. 1A) and weak biofilm producer Streptococcus oralis (graded 2+; Fig. 1B).
In this study, we attempted to isolate and characterise oral bacterial species from dental caries patients and caries-free individuals with no current oral disease. Table 1 shows the bacterial species isolated along with their source of isolation. Culturing of bacteria from oral samples on blood agar plates and chocolate agar plates often produced more than one type of colonies. Selected colonies were then sub-cultured onto fresh plates and used for MALDI–TOF–MS-based identification of bacteria. Most of the isolates belonged to the genus *Streptococcus*. This is in agreement with the previous reports that *Streptococcus* spp. are one of the most frequently isolated bacteria from oral cavity, and the common species are *Streptococcus salivarius*, *Streptococcus mutans*, *Streptococcus rattus* and *Streptococcus sobrinus*. Although *S. mutans* is established as the most common bacterial species causing dental caries, none of the samples analysed (either from patients with dental caries or caries-free subjects) yielded *S. mutans*. However, it is important to note that streptococcal species other than *S. mutans* such as *S. salivarius*, *S. sobrinus* and *Streptococcus parasanguinis* are also implicated in dental caries especially when *S. mutans* is absent. In this study, we identified several less common bacterial species both from patients with dental caries and caries-free subjects. An extensive survey of scientific literature showed that the *Streptococcus* species marked with asterisk (*) in Table 1 are isolated for the first time in Saudi Arabia.

Dental plaque is a biofilm of oral bacteria which when not cleared may lead to the development of dental caries. We used crystal violet dye binding biofilm assay which is a widely used method for biofilm detection and quantitation. We observed that the *Streptococcus* species isolated from oral samples produced varied amount of biofilm which ranged from 1+ to 4+ in arbitrary units. *Streptococcus salivarius* (3 strains), in general, produced higher amount of biofilm, irrespective of whether they were isolated from healthy individuals or patients with dental caries (Fig. 2). *Streptococcus*
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bacterial species (4 of which are

In conclusion, we reported the isolation of seven oral

35 oral samples (10 from patients with dental caries and

25 caries-free subjects) and 17 samples yielded identi-


**REFERENCES**


**CONCLUSION**

In conclusion, we reported the isolation of seven oral bacterial species (4 of which are *Streptococcus* spp.) for the first time from Saudi population. However, the biological significance of these oral bacterial species was not the focus of this study, and it remains to be evaluated in large-scale controlled studies.


