Pathogenic Bacteria Predominate in the Oral Cavity of Malaysian Subjects

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Abstract: This study identifies the major pathogens that constitute the normal oral flora of Malaysians and elaborates on their pathogenic potential. Major oral microorganisms identified by their 16S rDNA sequences include Streptococcus pneumoniae, Staphylococcus aureus, Streptococcus mitis and Neisseria subflava in the gingival crevice, Streptococcus oralis, Rothia mucilaginosa and Kingella oralis on teeth surface, Streptococcus infantis, Streptococcus pseudopneumoniae, Actinomyces viscosus on tongue surface. Organisms identified non-pathogenic included Pseudomonas aeruginosa in the gingival crevice, Streptococcus sanguis on teeth surface and Lautropia sp. on tongue surface. The presence of pathogenic organisms may have profound implications on the health of individuals harbouring them. Knowledge of the type of bacteria that inhabit the oral cavity is important in predicting and preventing not only dental diseases but also the associated systemic complications caused by them.

Key words: Systemic infection, microbes, biofilm

INTRODUCTION

Approximately seven hundred microbial species in the biofilm of the oral cavity are nourished with nutrients and provided with a conducive habitat. In addition, the dental plaque provides a cocoon for them, while the dextran they secrete allow them to bind to tooth surfaces. In species like Streptococcus mitis and Streptococcus sanguis combined there is good correlation between in vitro binding properties and cariogenicity (Willecox et al., 1985). The non-shedding tooth surface also provides for development of a complex ecosystem in the mouth.

In the oral cavity, the bacterial population is a result of the dynamic relationship between pathogens and commensals (Socransky et al., 2002). In the last decade, it has become increasingly clear that oral diseases, especially periodontitis are associated with and independent risk factors for systemic conditions such as cardiovascular disease, osteoporosis, diabetes mellitus and infection in other body sites (Renvert, 2003; Meurmann et al., 2004).

Commensal microorganisms found in the oral cavity rarely cause invasive infections except in immunocompromised adults or children. However, nonbeneficial microorganisms have direct link to dental caries, periodontal disease and halitosis. In immunocompromised hosts however, these microorganisms can cause opportunistic infections and systemic diseases. Oral bacteria have been implicated in bacterial endocarditis, aspiration pneumonia, osteomyelitis in children, preterm low birth weight, coronary heart disease and cerebral infarction (or stroke). The incidence of bacteremia following dental procedures has been well documented (Li et al., 2000). The major oral diseases are caused by disruption of homeostasis in the polymicrobial activity.

The type and distribution of oral microorganism in the biofilm vary with dietary and cultural habits and the health of individuals (Bowden and Li, 1997). A good understanding of the types and characteristics of the microbes of the oral cavity would be useful in managing infections caused by them. Knowledge of the susceptibility or resistance of these species to specific antibiotics will enhance therapeutic efficacy.

MATERIALS AND METHODS

This study was conducted between December 2007 and November 2008 in the Faculty of Science and Medicine of the University of Malaya.

Sample collection: Bacterial species were identified by their 16S rDNA sequences. Bacteria were collected using...
either the Gracey curette (teeth surface and gingival crevice) or cotton swab (tongue). Swabbed samples were suspended in Reduced Transport Fluid (RTF) and diluted 10, 10^2 and 10^3 times. Diluted samples were plated in duplicate on Columbia blood agar plates and incubated aerobically at 37°C for 3-5 days. Distinct colonies (2-3 colonies of apparently same morphology) were selected and streaked separately on agar plates. Single colonies obtained were characterized by gross (colour and shape) and microscopic morphology as well as by gram staining.

DNA preparation: The genomic DNA of pure cultures was prepared using i-genomic DNA extraction mini kit (INtRON Biotechnology, Seongnam). Extracted DNA was evaluated for both quality and quantity by agarose gel (1%) electrophoresis.

PCR and sequencing: Universal primers for 16S rDNA (forward: 5'-AGA GTT TGA TCA TGG CTC AG and reverse: 5'-TAC GCC TAC CTT GGT ACG ACTT) were used for PCR amplification. PCR conditions were: initial denaturation at 94°C for 5 min, denaturation at 94°C for 1 min, annealing at 52°C for 1 min, extension at 72°C for 1.5 min and final extension at 72°C for 10 min. PCR reaction mixtures (20 μL total) contained 2 μL of 10x PCR buffer, 2 μL of dNTP mix (2.5 mM each), 1 μL of each primer (10 pmol), 50 ng of DNA template, 0.5 μL of i-Taq™ DNA polymerase 5U μL, INtRON Biotechnology, Seongnam). The resulting PCR product was examined by electrophoresis on a 1.5% agarose gel and purified by PCR Quickspin kit (INtRON Biotechnology, Seongnam). DNA sequencing of the PCR product was performed by Macrogen, Seoul.

Species identification: Sequences were blasted to NCBI databases using BlastN and bacterial species identified on the basis of at least 98% similarity to database 16S RNA sequences. Cross reference was also made to the Human Oral Microbiome Database (HOMD) at www.homd.org.

RESULTS

Table 1 shows the percentage homology of the 16S rDNA sequences with NCBI Genbank sequence data. Species identity was established on the basis on at least 98% homology.

Accession numbers shown in the first column represent those allocated for our sequences, while those shown in the last column are sequences deposited in the Genbank.

Table 1: Accession number and homology with Genbank sequences

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<th>Accession No.</th>
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<th>Percentage</th>
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<td>Streptococcus australis</td>
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</table>

Fig. 1: Gram stain showing characteristics of Streptococcus pneumoniae

Pathogenic organisms

Gingival crevice: Streptococcus pneumoniae (Fig. 1), an alpha hemolytic and gram positive diplococcus is an inhabitant of the upper respiratory tract that can cause a number of life threatening diseases. Streptococcus pneumoniae is the main respiratory tract pathogen in otitis, sinusitis, bronchitis, meningitis, bacteraemia and community acquired pneumonia. Clinical infections with pneumoniae have been managed with antibiotic and vaccines although antibiotic resistant strains are common and many distinct serotypes exist. The bacteria have been found to have resistance to β-lactams, macrolides and fluoroquinolones (Pallares, 2003; Rossoni et al., 2008). Mutation at pnuemolyisin (Ply), Pneumococcal surface protein A (PspA) and Pneumococcal surface protein C (PspC) which are important for the colonization of the respiratory tract did
not prevent the animal model from infection. This study demonstrated that pneumococcal pathogenesis is complex and multifactorial (Ogurniyi et al., 2007). Mutants defective in growth rate remained capable of causing disease in animals as tested in mice and this study also showed that the pathogenesis is complex (Ferreiro et al., 2008).

*Staphylococcus aureus* (Fig. 2) are gram positive cocci as well as coagulase positive. Methicillin Resistant Staphylococcus Aureus (MRSA) are commonly found pathogens (Gould, 2007). *Streptococcus aureus* is frequently reported as a pathogen causing nosocomial infections, bacteraemia and endocarditis (Gould, 2007; Fowler et al., 2006). Frequent antibiotic administration can lead to selection of resistant strains over susceptible strains (Schentag et al., 1998).

*Streptococcus mitis* (Fig. 3) can be an important pathogen in adults and may cause infections including endocarditis and toxic shock-like syndrome.

*Neisseria subflava* (Fig. 4), a gram negative diplococcus may cause individuals to become susceptible to Streptococcal throat infections due to commensalism. In rare conditions it may cause Continuous Ambulatory Peritoneal Dialysis (CAPD) related peritonitis. This organism has also been implicated in meningitis, septicemia, endocarditis and endophthalmitis. The emergence of *N. subflava* isolates resistant to penicillin, fluoroquinolones and tetracycline have been noticed (Furuya et al., 2007). In response to ingested ethanol, *Neisseria* in the oral cavity could produce significant amount of carcinogenic acetaldehyde which is the risk factor for cancer of the upper aerodigestive tract (Muto et al., 2000).

**Teeth surface:** *Streptococcus oralis* (Fig. 5) are gram positive cocooid bacteria implicated in endocarditis.
Rothia mucilaginosa (Fig. 6) are normal oral flora which are catalase negative gram positive cocci. In rare cases it may cause bacterial meningitis and bacteremia. It has been reported that R. mucilaginosa cause late prosthetic joint infection and antibiotic prophylaxis is used for infection prevention of joint arthroplasties during dental procedures.

Kingella oralis (Fig. 7) gram negative rods or coccobacilli has been isolated from human dental plaque and may be associated with periodontis.

**Tongue surface:** *Streptococcus infantis* (Fig. 8) is an alpha hemolytic gram positive pathogen of the oral cavity.

*Streptococcus pseudopneumoniae* (Fig. 9) is genetically distinct from *S. pneumoniae* and is associated with Chronic Obstructive Pulmonary Disease (COPD). It is a non capsulated alpha hemolytic gram positive organism.

Actinomyces viscosus (Fig. 10) is gram positive filamentous bacteria occurring in dental plaque on cementum. In rare cases, this has been implicated in causing endocarditis.

**Non-pathogenic organisms**

**Gingival crevice:** *Pseudomonas aeruginosa* (Fig. 11), a gram negative aerobic rod shaped bacteria is found in the environment. Antibiotic resistance in this species is common.

**Teeth surface:** *Streptococcus sanguis* (Fig. 12) is associated with good oral health and lack of this organism indicates caries susceptibility. *Streptococcus sanguinis* has been implicated in native-valve infective endocarditis and bacteremia. These infections may be complicated by the increase of antibiotic resistance (Xu et al., 2007).
**Tongue surface:** *Lautropia* sp. (Fig. 13) notably *mirabilis* are commonly found in the oral cavity and are gram negative cocci (Rossmann *et al.*, 1998).

**DISCUSSION**

The development of the oral microbial community involves competition as well as synergy among the hundreds of species present in the oral cavity. Bacterial populations in the human oral cavity are constantly in a dynamic state of change. The need to profile and characterize these microorganisms using appropriate rapid detection methods can go a long way in developing future management strategies in clinical settings to enhance oral health in the Malaysian population.

Traditional methods for bacterial identification depended on phenotypic analyses which by themselves can cause misidentification of strains. Misidentification is usually either due to not all members of a given species may be positive for a particular identifying trait (Beighton *et al.*, 1991; Kilian *et al.*, 1989) or strains undergoing phenotypic shifts (Hillman *et al.*, 1989; Tardiff *et al.*, 1989). Due to its specificity and sensitivity, PCR based methods are currently widely applied for diagnostic purposes. Since 16S rDNA occur in multiple copies in the bacterial cell, it is a ideal target for taxonomic purposes. Additional targets such as 16S-23S rRNA intergenic spacer genes and species-specific virulence factors have provided some degree of specificity (Hoshino *et al.*, 2005).

Streptococci are the predominant bacterial organisms in the human oral cavity. In general, oral streptococci can be divided into six distinct groups: *Strep. gordonii*, *Strep. sanguis*, *Strep. oralis*, *Strep. mitis*, *Strep. parasanguis* and *Strep. crista* (Rudney and Larson, 1993; Coykendall,
CONCLUSION

The large number of pathogenic bacterial species present in the oral cavity of Malaysian subjects may indicate that the potential for the development of systemic diseases is always present. The non-pathogenic bacteria may be beneficial in maintaining the balance of species in the oral cavity.

REFERENCES


1989. Many species of this gram positive coccus are found in the oral cavity of Malaysian subjects. These include Strep. pneumoniae, Strep. pseudopneumonia, Strep. mitis, Strep. infantis, Strep. oralis. Non streptococci include Staphylococcus aureus, Neisseria subflava, Rothia mucilaginosa, Kingella oralis and Actinomyces viscosus. All of them may be considered important oral or systemic pathogens.

The occurrence of opportunistic pathogens in the oral cavity may be seen from the following aspects:

- **Oral hygiene**: Good oral hygiene would presumably allow the oral cavity to be colonized by a larger number of beneficial organisms and lower the number of pathogenic strains. Treatment by reduction of bacterial populations by antimicrobial or mechanical means is usually unsuccessful because the target bacteria are able to rapidly repopulate the oral cavity immediately after treatment (Burton et al., 2005)

- **Dental treatment procedures that introduce oral bacteria into the bloodstream**: It has been established that oral bacteria are found in blood following dental procedures (Bahrami-Mougeot et al., 2008) and the American Heart Association has issued guidelines and updates on procedures to prevent bacterial endocarditis (Wilson et al., 2007)

- **Probiotics**: The use of probiotics offer oral benefits (Caglar et al., 2005). One strategy employed is the depletion of oral bacteria by use of the antimicrobial chlorhexidine and repopulation of the oral cavity with beneficial organisms such as S. salivarius, a benign commensal probiotic (Burton et al., 2005)

- **Clinical management of oral cavity and systemic infections**: Li et al. (2000) have evaluated the status of oral infections as a causal factor for systemic diseases and have discussed mechanisms or links between oral infection and secondary systemic effects. Since both gram positive and gram negative bacteria can potentially cause systemic infections, any management strategy must take this into account. Furthermore, many bacteria are antibiotic resistant or have the potential to acquire resistance. The relationship between oral health and general health is bidirectional and complex (Johnson et al., 2006). Brook (2003) describes the prudent management of odontogenic infection and preventive strategies in a comprehensive article.

The oral cavity of Malaysian subjects also contains non pathogenic bacteria including Capnocytophaga granulosa, Strep. sanguis and Lautropia. These organisms are important in maintaining the proper ecology of the oral cavity. This may in part help to maintain good oral health.


