Determination of Aerobic Bacterial Composition of Dental Plaque Biofilms and Their Role in Oral health

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Received: 3 March 2015, Accepted: 25 March 2015

Abstract
Dental plaque is the biofilm found naturally on teeth and it is the etiologic agent in dental caries and periodontal diseases. A better understanding of bacterial communities found in biofilms provides opportunities for new methods to control biofilm formation. Therefore, control of the dental plaque biofilm is a major objective of dental professionals and critical to the maintenance of optimal oral health. The aim of this study was to isolate and identify aerobic bacteria from dental plaque biofilm on the external surface of the teeth and gums and to determine their role in the etiology of gingivitis and dental caries. Fifty nine samples of dental plaque from gingiva and teeth were collected randomly from patients attending Sebha dental clinic, Libya, males and females of different age groups. All samples were cultured on different media, conventional methods used for isolation and identification. Results revealed that 52 samples (88%) were positive for culture, and they were polymicrobial. Bacteria isolated were 110 species, 93 (84.5%) were gram positive composed of Streptococcus species 43 (39%), Lactobacillus 27 (24.5%), Staphylococcus 23 (21%) and 17 were gram negative (15.5%), they were E.coli 7 (6.4%), Enterobacter 6 (5.5%), Proteus 4 (3.6%). Isolates from males were 66 (60%) and females were 44 (40%), 61 (55.5%) of the isolates were from children (5-15) years old (44% were Streptococcus species and 25% were lactobacilli, Staphylococcus 18%) and 49 (44.5%) were from the age group (16-68) years old (28% were Streptococci and 21% were Lactobacilli). Most of the isolated bacteria (58%) was from patients who do not clean their teeth by daily brushing with toothpaste.

Keywords Dental plaque biofilms, composition, role in oral health

Introduction
The oral cavity is colonized by a diverse microflora. Several bacterial species have been implicated as causative agents of various oral diseases (Kulik et al., 2008). Dental plaque is an example of a microbial biofilm with a diverse microbial composition; it is present normally on teeth and gives benefit to the host, by protecting colonization by exogenous (Marsh, 1999). Numerous studies have been undertaken to determine the composition of the plaque microflora from diseased sites in order to try and identify those species directly implicated in causing pathology. (deSoet et al., 2000). Microbes in biofilms like plaque are in close to physical touch, and this can augment the chance of interactions, some of which can adapt the pathogenic prospective of cariogenic bacteria (Becker et al., 2002). Medical researches have revealed that caries is related with increases in the proportions of acidogenic and aciduric bacteria, especially lactobacilli and mutans streptococci (such as S. mutans and S. sobrinus), which are able to demineralizing enamel (Loesche, 1986). In addition, microbes of the oral cavity are taking advantages and causing infections in the head and neck (such as infections of the jaw bones or fascia, periapical abscesses, pulp infections) as well as new parts of the body (brain abscesses, cellulitis, endocarditis, meningitis, septicemia and osteomyelitis ). Thus, the control of oral bacteria is of paramount importance, and the proper use of appropriate antibiotics is an effective means of control (Chan et al., 1989). It is duty of dentist to
tell their patients that, periodontal disease and dental caries disease are infectious, caused from dental plaque biofilm accretion. All of these diseases need specific approaches for cure and treatment (Gurenlian, 2007). Although dental biofilm cannot be completely end, but its pathogenicity can be low through effectual oral hygiene measures (Mager et al., 2003). Antimicrobial approaches to prevent dental caries, including, minimizing plaque levels, in regular or particular cariogenic bacteria, dropping bacterial acid production by replacing carbohydrates in the food with sugar alternates, or by intrusive with bacterial metabolism with antimicrobial agents or fluoride (Marsh, 1999).

The purpose of this research was to recognize and isolate aerobic bacteria from dental plaque biofilm on the external surface of the teeth and gums and to determine their role in the etiology of gingivitis and dental caries.

**Methodology**

This microbiological investigation was conducted during the period of September, 2012 to March, 2013. A total of 59 dental plaque specimens were collected from patients attending Sebha Dental Clinic, Libya. The subjected patients were chosen randomly males and females, ranging in age from 5 to 68, not used antibiotics for the last one week and all patients have various dental and periodontal problems.

**Sample Collection and Isolation of Bacteria**

Sterile cotton swaps were used to collect bacterial samples from gingiva and teeth appropriately with complete aseptic precautions under the assistance of dentist, inoculated onto selective medium (blood agar and MacConkey agar). All the plates were incubated aerobically at 37°C for 24–48 hours. Identification was based on colony morphology, gram stain reaction, and biochemical tests including catalase, coaglase, oxidase, indole, methyl red and urease according to Barrows and Feltham (2003) Gram negative bacteria from the Enterobacteriaceae family were identified by using API-20E system (Analytical profile index bioMe’reux, France).

**Data Collection**

A questionnaire forms were collected back including age, sex, oral hygiene by means of tooth brushing, drugs uptake, oral health and dental problems and other data of each patient was noted.

**Results and Discussion**

In this study fifty nine samples of dental plaque from gingiva and teeth were collected randomly from patients attending Sebha dental clinic, males and females of different age groups. All samples were cultured on different media, conventional methods used for isolation and identification. Results showed that 52 samples (88%) were positive for culture, and they were polymicrobial, bacteria isolated were 110 species, 93 (84.5%) were gram positive composed of Streptococcus species 43 (39%), Lactobacillus 27 (24.5%), Staphylococcus 23 (21%) and 17 were gram negative (15.5%), they were E.coli 7 (6.4%), Enterobacter 6 (5.5%), Proteus 4(3.6%) (Table 1).

This is in agreement with other workers, Saini et al. (2003) in their study revealed that the commonest aerobe isolated was Streptococcus mutans in dental caries, Staphylococcus aureus on gingivitis. Sharma et al. (2011) found that the highest number of aerobic organisms isolated in dental caries, gingivitis and periodontitis were Streptococcus mutans and Staphylococcus aureus. Simonović et al. (2002) in their study in Serbia isolated the same species from dental plaque. These bacteria are opportunistic pathogens, found commonly as members of the resident flora of persons without caries and expressing their pathogenicity only under specific environmental conditions. Streptococcus mutans and Streptococcus sobrinus, two species of the ‘mutans streptococci’ are the most significant in human caries and studies of the microbial ecology of caries have been directed principally at these species. There is also a strong association between Lactobacillus spp. and caries but little is known of the relative significance of the different species (Bowden, 2000), aerobic lactobacilli were regular in dental caries. The results from several studies suggest that other bacteria can also become dominant in plaque community and be associated with demineralization and for formation of a caries lesion (Scheie et al., 1996). It is well accepted that the microflora of lesions in teeth and tooth roots is extremely complex and may vary at different sites (Bradshaw and Marsh, 1998).

In our study we found that isolates from males were 66 (60%) and females were 44 (40%).
Table 1

Number and percentage of isolated bacteria.

<table>
<thead>
<tr>
<th>Gram Negative Bacteria</th>
<th>Gram Positive Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>No.</td>
</tr>
<tr>
<td>E.coli</td>
<td>7</td>
</tr>
<tr>
<td>Enterobacter</td>
<td>6</td>
</tr>
<tr>
<td>Proteus</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
</tr>
</tbody>
</table>

This is in difference to the results of other researchers, when dental caries rates are reported by females, sex are typically found to exhibit more prevalence (Lukacs and Largaespada, 2006).

Among the different age groups studied we found that 61 (55.5%) of the isolates were from children (5-15) years old (44% were Streptococcus species, 25% were lactobacilli and Staphylococcus 18%) and 49 (44.5%) were from the age group (16-68) years old (28% were Streptococci and 21% were Lactobacilli) (Table 2). This is similar to the results obtained by Whab and Alkabi (2011) who revealed that Streptococcus and Lactobacillus were dominant in children in dental plaque isolates. This is may be due to the fact that the habit of sugar consumption in the form of chocolates and other sugar containing food is comparatively higher among kids. The daily eating of fermentable dietary sugars, or damaged saliva flow, making persistent forms of low pH in the biofilm, which choose for these cariogenic bacteria (Marsh, 2010).

Table 2

Number and percentage of isolated bacteria according to age sex and teeth cleaning

<table>
<thead>
<tr>
<th>Age</th>
<th>Percentage (%)</th>
<th>Number of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-15</td>
<td>55.45</td>
<td>61</td>
</tr>
<tr>
<td>16-30</td>
<td>29.25</td>
<td>32</td>
</tr>
<tr>
<td>31-50</td>
<td>7.2</td>
<td>8</td>
</tr>
<tr>
<td>51-68</td>
<td>8.1</td>
<td>9</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>60</td>
<td>66</td>
</tr>
<tr>
<td>Females</td>
<td>40</td>
<td>44</td>
</tr>
<tr>
<td>Teeth cleaning</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do not clean teeth</td>
<td>53</td>
<td>58</td>
</tr>
<tr>
<td>Clean teeth 2 times/day</td>
<td>35.5</td>
<td>39</td>
</tr>
<tr>
<td>Clean teeth 3 times/day</td>
<td>2.7</td>
<td>3</td>
</tr>
</tbody>
</table>

Various studies have established a clear link between caries and the quality of life of both children and adults (Filstrup et al., 2003). As teeth retention in the population increases, dental caries has become a burden for ageing adult’s worldwide (Kirkevang et al., 2011). In developing countries, this health issue is widespread in young as well as adult populations. For example, the dental caries prevalence in Tunisia was shown to vary between 48 and 58% in the young population (6-15 years old) (Abid, 2004).

Also we observed that most of the isolated bacteria 58 (53%) was from patients who do not clean their teeth (Table 2). Daily brushing with toothpaste that contains antibacterial agent, has been shown to reduce the growth of oral bacteria and the formation of plaque (Gaffar et al., 1995). While dental biofilm cannot be eradicated, it can be minimized and managed through daily oral care. A routine mechanical oral hygiene procedure, including interdental cleaning and tooth brushing are main solutions to controlling biofilm accumulation (Gurenlian, 2007). A better understanding of bacterial communities found in biofilms, such as its interactions and diversity between cells, provide chance for new technique to control biofilm formation (Wade, 2010). An accurate diagnosis of periodontal disease severity is essential for selecting an appropriate treatment and maintenance strategy for a given patient (American Academy of Periodontology, 1999, Gaffar et al., 1995). The optimal strategy to eliminate dental biofilm from the oral cavity has four dimensions: physical removal of dental biofilm; destruction of the remaining bacteria using antimicrobial agents; routine oral hygiene habits; and patient education. Each day removal of supragingival plaque decreases gingival inflammation and also manages the amount of subgingival plaque. It also can
drastically decrease the proportion of identified pathogens. (Socransky and Haffajee, 2002).

**Conclusion**

Dental biofilm is a major etiologic feature for the most commonly occurring oral diseases, periodontal diseases and dental caries. Although the dental biofilm cannot be purged, it can be managed with broad mechanical and chemotherapeutic oral hygiene approaches. Communicate to patients that cure from periodontal disease by doing daily interdental cleaning, antimicrobial mouth rinses and brushing. Careful isolation of bacterial pathogens is important in the treatment of orodental infections.

**Acknowledgements**

Special thanks to Sebha dental clinic administration and the laboratory technicians of the Department of Medical Laboratory Sciences, Faculty of Engineering and Technology, Sebha University for their help and cooperation.

**References**


