Bacterial contamination of toothbrushes with comparison of healthy and dental patients

Rana Mohammad Abd-ulnabi
Department of Pharmacology and Clinical Lab. Sciences
College of Pharmacy - University of Basrah - Basrah - Iraq

Abstract

Twenty four normal toothbrushes were tested for adult persons, 12 brushes used by healthy individual and 12 brushes used by patient of oral infection (gingivitis or periodontitis) each brush was used for at least 5 weeks period.

Both brushes of two groups were colonized by large number of organisms ranged from $0.2 \times 10^2$ to $3.5 \times 10^2$ C.F.U/ml on healthy individual brushes and from $2.8 \times 10^2$ to $5 \times 10^2$ C.F.U/ml on patient brushes.

Each brushes of healthy individual yielded various types of organisms as Pseudomonas, Staphylococcus epidermidis, Staph. aureus, Gram positive rods and yeast but most brushes of patients yielded one type of organisms.

T-test analysis appeared that there were high significant difference at ($P < 0.05$) between brushes of two groups in the total mean of different organisms that isolated from them.

Pseudomonas recorded highest proportion (57% of total organisms isolated on all brushes of two groups; 83% of brushes) followed by Staphylococcus (36% of total isolated organisms; 58% of brushes) Gr+ve recorded lowest proportion(3% of total isolated organisms; 33% of brushes).

Staph. epidermidis, Staph. aureus could isolate from brushes of patient in 6 days after brushing while Pseudomonas isolated after 3 days.

This study demonstrated Staphylococcus and Pseudomonas as pathogen agent that cause oral infections and conclude that toothbrushes may be as a source of opportunistic pathogen such these microorganisms by wrong storing ways or by the same infected person.
Introduction

The human oral cavity is colonized by a larger variety of bacteria flora than any other anatomic area. More than 700 species of bacteria have already been identified 400 of which were found in the periodontal pocket adjacent to teeth (Abraham et al., 1990). Organisms not normally associated with oral flora also have been isolated from toothbrushes, including enterobacteria, Pseudomonas (Sammons et al., 2004). So the infectious microorganisms remaining on the brush can reinfect our mouth teeth again, some of them can even spread to the rest of our body and cause serious health problems, including heart disease, stroke, arthritis, haematogenous, bacterimia and chronic (Warren et al., 2001; Sammons et al., 2004). There are many ways allows the bacteria bread and grow on toothbrushes, spray from flushing toilet, adamp environment, a single toothbrush can be the breeding ground for billions of bacteria (Abraham et al., 1990).

There are attempt to reduce bacterial survival time, deter colonization and inhibit biofilm formation by toothbrushes containing antibacterial agent have been developed and methods for sterilization of brushes devised (Caudry et al., 1995; Neal & Rippin, 2003). Particular attention was paid to Staphylococci and Pseudomonas like organisms as both of these are opportunistic pathogens responsible for many nosocomial infections and because Pseudomonas are also resistant to many disinfectants in toothpaste including triclosan (Warren et al., 2001). The aim of this study was to investigate and compare bacterial population on toothbrushes.

Materials and methods

Collection of samples

In this study (24) toothbrushes for adult individuals brushed with them for at least 5 weeks have been tested, (12) of them were for healthy individual (H.I) and (12) samples from adult person suffering from gingivitis or periodontitis as their doctors diagnosis.

Isolation of organisms

Toothbrush of every person were rinsed in tap water and transported to the laboratory in sterile bag, according to Sammons et al. (2004) handle of brush was cut off using a heat sterile scissors, head of the brush was then soaking in 10 ml of sterile tryptone soya broth (TSB), for 60 min, followed by vortex mixing for 1 min and make swabbing to dislodge suspected adherent bacteria.

The bacterial suspension was one fold diluted for $10^{-1}$ and (0.1 ml) of broth plated by pipette into tryptone soya agar (TSA), as non-selective media and into MacConkey, Manitol salt agar and Sabouraud’s dextrose agar to isolate enterobacteria, Staphylococci and yeasts, respectively, plates were incubated aerobically at 37°C for 24-48 h.
Identification:-
A total viable counts of bacterial population were enumerated, colony colour, morphology and Gram’s stain was performed for each isolates.

A. Gram positive cocci of Manitol salt agar were further identified as Staphylococcus aureus and Staphylococcus epidermidis by several biochemical tests:-
1. Catalase test (Collee et al., 1996).
2. Oxidase test (Benson, 2002).
3. Coagulase test (Collee et al., 1996).
5. Deoxyribonuclease (DNAase) test (Collee et al., 1996).
6. Carbohydrates fermentation test (Benson, 2002).

B. Gram negative bacilli on MacConkey plates were identified as following:

a. Gram negative, non lactose fermenting, oxidase positive colonies were considered as Pseudomonas spp
b. Gram negative, lactose fermenting, oxidase negative colonies were considered as Coliform spp.

Survival of isolates on toothbrushes:-
After culture and characterization bacteria on brushes were diagnosed into of two groups, patient’s brushes that labeled with (13,15,22) were selected for bacterial survival of Staph. epidermidis, Staph. aureus, Pseudomonas respectively. Each person used these brushes provided with three new sterile brushes for brushing for at least 3 weeks then storage them in sterile bag for various period (24 h, 3 days, 6 days) (Sammons et al., 2004).

Statistical analysis: Student t-test analysis was applied to determine the significance of differences at (P<0.05) between brushes types in total numbers for each types of organisms and in the total means.
Result and Discussion

Table 1: The total number of organisms on each brush of two groups.

<table>
<thead>
<tr>
<th>H.I brushes</th>
<th>Total number of organisms C.F.U/ml</th>
<th>P. brushes</th>
<th>Total number of organisms C.F.U/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1H</td>
<td>1.3×10^2</td>
<td>13P</td>
<td>3.5×10^2</td>
</tr>
<tr>
<td>2H</td>
<td>0.7×10^2</td>
<td>14P</td>
<td>1.5×10^2</td>
</tr>
<tr>
<td>3H</td>
<td>0.3×10^2</td>
<td>15P</td>
<td>4×10^2</td>
</tr>
<tr>
<td>4H</td>
<td>0.5×10^2</td>
<td>16P</td>
<td>3×10^2</td>
</tr>
<tr>
<td>5H</td>
<td>0.2×10^2</td>
<td>17P</td>
<td>3.3×10^2</td>
</tr>
<tr>
<td>6H</td>
<td>0.4×10^2</td>
<td>18P</td>
<td>2.7×10^2</td>
</tr>
<tr>
<td>7H</td>
<td>1.7×10^2</td>
<td>19P</td>
<td>2.8×10^2</td>
</tr>
<tr>
<td>8H</td>
<td>3.5×10^2</td>
<td>20P</td>
<td>5×10^2</td>
</tr>
<tr>
<td>9H</td>
<td>0.2×10^2</td>
<td>21P</td>
<td>3.2×10^2</td>
</tr>
<tr>
<td>10H</td>
<td>1.3×10^2</td>
<td>22P</td>
<td>4.5×10^2</td>
</tr>
<tr>
<td>11H</td>
<td>0.5×10^2</td>
<td>23P</td>
<td>4.2×10^2</td>
</tr>
<tr>
<td>12H</td>
<td>1.1×10^2</td>
<td>24P</td>
<td>2.8×10^2</td>
</tr>
</tbody>
</table>

P= Patient's sample          H.I= Healthy individual's sample

Table 2: The average total numbers for each microorganism and the total mean.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Average total numbers (C.F.U/ml)</th>
<th>p.V (p&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy individual's brushes</td>
<td>Patient's brushes</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>5.9×10</td>
<td>18.75×10</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>2×10</td>
<td>13.66×10</td>
</tr>
<tr>
<td>G+ve rod</td>
<td>1.25×10</td>
<td>0.25×10</td>
</tr>
<tr>
<td>Yeast</td>
<td>0.5×10</td>
<td>1×10</td>
</tr>
<tr>
<td>Total mean</td>
<td>9.75×10</td>
<td>33.57×10</td>
</tr>
</tbody>
</table>

* = There were significant difference, N.S = No significant difference, ** = There were high significant difference
The total number of brushes that tested were 24. All brushes of two groups was yielded microbial colonies. There were variable in numbers of organisms on each brush but both brushes of healthy individual and patient were colonized by large number of organisms ranged from $0.2 \times 10^2$ to $3.5 \times 10^2$ C.F.U/ml on H.I brushes and from $1.5 \times 10^2$ to $5 \times 10^2$ C.F.U/ml on P. brushes, table 1. The number of bacteria on brushes of patient was highest than those of H.I ones, these difference in bacterial load belong to presence oral inflammations (Taji et al., 1998) table 1.

The total numbers of different types of isolates on brushes of each group was calculated, t-test showed there were significant difference at $p<0.05$ between brushes of two groups in the average total numbers of Pseudomonas and Staphylococcus, while no significant difference appeared for Gr+ve rod and yeast. From the other hand t-test also appeared high significant difference between the total mean of all types of organisms that isolated from brushes of each group, table 2, Fig 1.

All brushes of H.I yielded a mixed population of organisms, with one to four different types of colony on each brush, while most brushes of patients yielded one type of bacteria Pseudomonas or Staphylococcus, but with large number comparison with that on H.I brushes. This may be due to the competition between different organisms and bacterial pathogens have evolved specific virulence factors that allow them to impair or kill other microbes (Nester et al., 2001).

Yeast were approximately isolated from half brushes of H.I in low numbers as flora, while from patient’s brushes yeast isolated only from one case which was relatively in large number, it is seem the pathogen that cause infection. Pseudomonas was recorded the highest proportion of total organisms isolated on brushes of H.I and patient (61%, 56%) respectively followed by Staphylococcus (21%, 40%), always store toothbrush in closed container not in ventilated environment and keeping it in toilet place, causing of presence of these bacterial types on brushes because of these moisture environments is more stabilized when the brush is not aired (Caudry et al., 1995). Comparison between relative proportion of different microorganisms that isolated from brushes of each group are shown in fig. 2.
Significant difference at $p < 0.05$

Fig 1: the total mean of all isolates on brushes of two groups

Fig 2 Percentage of microorganisms isolated from brushes of H.I and patients.
Table 3: Proportion of microorganisms isolated from all brushes and Percentage of positive brushes:

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Percentage of positive brushes</th>
<th>Proportion of isolates</th>
<th>Range (C.F.U/ ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas</td>
<td>83%</td>
<td>57%</td>
<td>1×10 - 4.5×10^2</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>58%</td>
<td>36%</td>
<td>1×10 - 4×10^2</td>
</tr>
<tr>
<td>Staph.epidermidis</td>
<td>79%</td>
<td>24%</td>
<td>1×10 - 3.5×10^2</td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>21%</td>
<td>12%</td>
<td>1×10 - 4×10^2</td>
</tr>
<tr>
<td>Gr+ve rod</td>
<td>33%</td>
<td>3%</td>
<td>1×10 - 5×10</td>
</tr>
<tr>
<td>Yeast</td>
<td>29%</td>
<td>4%</td>
<td>1×10 - 2×10</td>
</tr>
</tbody>
</table>

Out of the total (24), 14 brush yielded bacterial growth on Manitol salt agar (58% of brushes); 3 brushes (21%) showed a growth of Staphylococcus aureus; 11 brush (79%) showed a growth of Staphylococcus epidermidis, table 3.

Several previous studies have reported the isolation of Staphylococcus from toothbrushes (Alshayeb & Al-Ebrahim, 2008; Gabe et al., 2011; Malmberg et al., 1994; Taji & Rogers, 1998; Verran & Gilmartin, 1996). People can get Staphylococcal infection from contaminated objects, and can be spread from one area of the body to another if some one touches the infected area, share things like brush, towels, clothing, warm, humid environments can contribute to Staphylococcal infection.

Both Staph.epidermidis isolates were cultured from both brushes of two groups, but Staph.aureus isolates were cultured from only brushes of patient and just in three samples, it found in two samples of them with presence of large number of Pseudomonas. The result showed that Staph. epidermidis was that cause oral infection in three cases of patient individual these ensure the potential pathogenicity of it. Staph.aureus have also been recorded among isolates from toothbrushes in (Gabe et al., 2011; Smith et al., 2003; Taji & Rogers, 1998).

Proportion of Staph. epidermidis (24%) of total organisms was larger in two time than these recorded by Staph.aureus (12%), table 3. These results differences compatible with (Alshayeb & Al-Ebrahim, 2008) which recorded (26.6%), (20%) for S.epidermidis, S.aureus respectively.
Pseudomonas, Staphylococcus were isolated from 83% 58% of all brushes respectively, it was more than 16%, 48% reported by (Sammons et al., 2004).

Pseudomonas are known to be resistant to triclosan (antibacterial agent is added to toothbrushes) (Van Delden & Iglewski, 1998) may be for these reason Pseudomonas recorded highest proportion of (57%) of total organisms isolated on all brushes of two groups. Coliforms were not isolated in this study, although they isolated in other studies in different countries (Alshayeb & Al-Ebrahim, 2008; Sammons et al., 2004).

Yeasts were identified in 29% of brushes, Sammons et al., 2004 were identified no yeast and Streptococcus were rarely because of the aerobic culture condition, in this study could not isolate Streptococcus for the same reason.

Table 4: Survival of S. aureus, S. epidermidis and Pseudomonas isolated from selected brushes

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>No. Sample</th>
<th>Total No. of bacteria</th>
<th>24 hr</th>
<th>3 day</th>
<th>6 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. epidermidis</td>
<td>14</td>
<td>4 × 10^2</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>S. aureus</td>
<td>12</td>
<td>3.5 × 10^2</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>22</td>
<td>4.5 × 10^2</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Numbers of S. aureus, S. epidermidis reduced by a factor of approximately 10 after 24 hr of storage and results of culture showed that growth of the two species were still viable after 6 days of storage, so same results recorded by (Sammons et al., 2004).

Pseudomonas also showed a decline in numbers of viable organisms, survivors were present after 3 days on brushes. But no growth at 6 days as shown table 4.

The persistence of viable Staphylococcus on drying toothbrush, especially in the humid atmosphere of toothbrush holder, is not surprising, since they can survive on hospital fabrics for several days (Neely & Maley, 2000) and both Staphylococcus and Ps. aeruginosa have been shown to survive in dried up films on non-nutrient surfaces, cotton and blood protein coagulum for several months (Smith et al., 1996). Most reports were showed bacterial colonization on toothbrushes by composition biofilm on them (Quirynen, 2003).

Several previous studies recommends healthy individual changing toothbrushes every three months, Sick children or adults should replace their toothbrushes as soon as possible to prevent re-infection or infection of another person (Glass & Lare, 1986).
REFERENCES


Bacterial contamination of dental implants and toothbrushes.


مقارنتها لـ 5 أسابيع من

عينات فرش المجموعةين أُستخدمت بأعداد كبيرة من الجراثيم تراوحت نتائج العدد البكتيري في عينات 2 (عينة) (12) منها لأشخاص أصحاء (12)

عينات فرش المرضى المكونه للعديد المستمر 2/10^2 × 3/10^2 الوحدات المكونة للعديد المستمر / وفي عينات فرش المرضى كانت 2/10^2 × 5/10^2 المكونه للعديد المستمر / تميزت عينات فرش الأصحاء بنمو أنواع مختلفه من الجراثيم المكورات العنقودية الذهبيه و المكورات العنقودية البيضاء وعصبات موجبة الغرام في حين عزل نوع واحد من الجراثيم أغلب عينات

بينت نتائج التحليل الاحصائي أن هناك فرق معنوي ممهم (P<0.05) بين فرش المجموعتين في الجراثيم المعزولة لجراثيم الفم سجلت جراثيم نسبة (57%) من مجموع الجراثيم المعزولة عينات الفرش تلتها جراثيم العقوديات (36)

عانات موجبة الغرام أقل نسبة عزل (3)

أظهرت كلا المكورات العنقودية الذهبيه و المكورات العنقودية البيضاء القدرة على البقاء على عينات فرش الأسنان المرضي المنتظم بعد 6 أيام من التفريش أو الحفظ في حين سجلت عينات فرش الأسنان الملونة كمستودع لعدد من الجراثيم الانتهائية العقوديات 3 أيام من التفريش.بينت هذه الدراسة