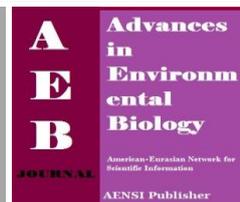




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Investigations into the Microbial Contamination of Toothbrushes Isolated from Riyadh, Saudi Arabia

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ABSTRACT

Aim: The aim of this study is to isolate and identify the bacteria and fungi species contaminating toothbrushes and their sensitivity to antibiotics in Riyadh, Saudi Arabia. **Sample:** The sample consisted of toothbrushes that were randomly collected from 20 healthy female volunteers and used for at least 3 weeks. **Materials and methods:** The toothbrushes were classified into three groups, each of which were processed differently to obtain the highest microorganism yield. The samples were then inoculated on the suitable media and incubated. Finally, antibiotic resistance was tested. **Results:** In this study, 90% of the toothbrushes had some type of microbial growth on them. *Staphylococcus sp.* was the most common with 25% of the toothbrushes. Twenty percent of the samples showed growth of *Lactobacilli sp.*, *Streptococcus sp.* were found in 5% of the samples. *Yeast cell sp.* were found in 20% of the samples. Three samples developed *Pseudomonas fluorescens* equated to 15%. The percentage of bacteria isolated from toothbrushes stored in bathrooms was 65%. Brushes stored outside the bathroom had a contamination percentage of 25%. Out of all the isolates, *Pseudomonas fluorescens* showed the greatest degree of resistance.

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INTRODUCTION

Oral health is an integral part of general health. It directly and indirectly reflects the overall well being of an individual. Thus, maintaining oral hygiene is a crucial factor of healthy living. Oral diseases can be greatly controlled by reducing the microbial load in the oral cavity. This can be achieved by maintaining proper oral hygiene. Brushing teeth is the main mode of oral hygiene practice and preventing dental diseases [11]. Toothbrushes may become contaminated with microorganisms [12,21,22]. These microorganisms may originate not only from the oral cavity but also from the environment where the toothbrushes are stored. This contamination may lead to reinfection of a consumer by toothbrushes that harbor pathogenic microorganisms [8]

Unfortunately, proper care of toothbrush is often neglected as toothbrushes are usually kept in bathrooms that harbor millions of microorganisms. This is mainly due to a lack of awareness regarding proper toothbrush maintenance. Therefore, The survival of microorganism on a toothbrush after brushing presents a possible mode of re-contamination upon a second usage [23].

The prolonged use of a toothbrush facilitates contamination by various micro-organisms such as *Streptococcus*, *Staphylococcus* [21] and *lactobacilli* (Fernandez & Cesar, 2006). Such microorganisms may cause dental diseases as well as be source of infection for more serious conditions such as infective endocarditis [3].

Some of the microorganisms isolated from used toothbrushes were *streptococcus mutans*, *staphylococcus aureus*, *pseudomonas*, *lactobacillus*, *klebsiella*, *candida*. These species were isolated in from toothbrushes kept in the bathrooms that lacked a toilet seat. *Escherichia coli* was found in toothbrushes kept in the bathrooms with a toilet [9]. In 2008, Saravia and colleagues conducted an in vitro study to assess the viability of streptococcus species on toothbrush bristles relative to the time required for them to dry [17]. Members of *Streptococcaceae* were found belonging to the following species: *Streptococcus pyogenes*, *S. mutans*, *S. mitis*, *S. oralis*, *S. sobrinus*, *S. viridans*, *S. salivarius*, *S. sanguis*, *aerococcus viridans* and *A. viridans*. Their study found that *S. mutans* were more frequently isolated from children toothbrushes and adult toothbrushes constituted the most frequent source of *staphylococcus aureus* and *S. epidermidis*. Researchers were also able to recover *Escherichia coli*, *Pseudomonas sp.* and *Enterococcus sp.* [2].

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In regards to fungal species, studies confirmed that all fungal species isolated from the toothbrushes were opportunistic and therefore may cause health problems mainly in immunocompromised patients. The species most frequently found were: *Candida albicans*, *Aspergillus niger*, *Penicillium citrinum*, *Geotrichum candidum*, *Aspergillus fumigatus* and *Cladosporium oxysporum* [14].

It is a well known fact that fluoride-containing toothpastes have a significant effect on the initiation and progression of dental caries [4]. Hays undertook a study in 2006 to determine which toothpaste brand (Aquafresh, Colgate, or Crest) repressed bacterial growth most effectively. The results of that study showed that there was statistically significant difference among the three brands of toothpaste. Thus, no one toothpaste brand was more effective in repressing bacterial growth than the others [7]. The effect of the tooth whitening components of toothpaste (a recent trend in the toothpaste industry) on microorganism growth is largely unknown.

The aim of this study is to isolate and identify the bacteria and fungi species contaminating toothbrushes and their sensitivity to antibiotics in Riyadh, Saudi Arabia.

MATERIALS AND METHODS

The samples were randomly collected from 20 healthy female volunteers aged 18 to 25 years old attending Princess Noura Bint AbdulRahman University in Riyadh, Saudi Arabia. It was confirmed that each toothbrush was used for at least 3 weeks before sample collection.

Brushes were collected in sterile bags. The toothbrushes were classified into three groups:

Group A (included brushes 1-10). Samples were processed as follows: The upper part of the toothbrush including the head and the bristles was cut. It was then placed in sterile container filled with normal saline 10% solution enough to cover the whole of the brush head. In order to insure that all residue of the brush was within the solution, each brush head was rubbed against a sterile cotton swab over the container. The container was sealed with the brush head inside and vortexed for 2 minutes. The brush head was allowed to soak in the solution for 10-15 minutes. To isolate the microorganisms, a sterile swab was used to isolate the sample from between the brushes bristles. Blood agar, mycological agar and MacConkey agar plates were inoculated with the swab samples. Also 1 ml of the solution in which the brush head had soaked was transferred on to the plates. Plates were incubated for 24-48 hours at 37°C.

The five toothbrushes of Group B (including samples number 11 to 15) were also collected in sterile plastic bags. These toothbrushes were processed as follows: Parts of the bristles were cut with sterile scissors. Those bristles were cultured and quantified by placing them directly on the following media: blood agar plates, nutrient agar plate and Sabouraud's dextrose agar plate. Plates were incubated for 24-48 hours [18].

Group C (included samples 16-20). This group were aimed to preserve the greatest number of microorganism, particularly fungi. Samples were processed as follows: the top part of five toothbrushes was cut then placed directly onto the following media: nutritious agar plates. Plates were incubated for 24-48 hours at 37°C [8].

Bacterial Isolation and Identification:

After the incubation period, the number of colony forming units was counted using the CFU/mL. The microorganisms were identified based on the different types of colonies. Colony morphologies were recorded and purified to obtain pure colonies for the identification purposes. Each representative colony was gram-stained and examined for cell morphology and gram reaction under a light microscope [9].

Preparation of Bacterial Suspension for Antibiotic Sensitivity Studies:

All bacterial strains were grown in BHI agar and incubated at 37°C overnight. The colonies were then harvested and dispersed into 0.85% sterile saline until it visually matched the McFarland 0.5 turbidity standard for use in antibiotic sensitivity test.

Antibiotics Sensitivity Test:

The test was conducted by the disc diffusion method using the Kirby-bauer test. The antibiotics used in this study were Ampicillin (10 µg), Cefuroxime (30 µg), Amoxicillin/clavulanate (30 µg), Ceftriaxone (30 µg) and Cefepime (30 µg). All antibiotic discs were purchased from Oxoid Chemical Co., England. The test cultures were prepared as mentioned above and swab evenly on the Mueller hinton agar using a sterile cotton swab and allowed to dry for 5 minutes using a fine forceps, antibiotic discs were placed onto the agar firmly and plates were incubated at 37°C for 18 to 24 hours. Susceptibility of the bacteria towards antibiotic was observed as inhibited zone surrounding the discs. The diameter of the inhibited zone was translated to sensitive (S) intermediate (I) or resistant (R) (Table 2) [8].

Results:

In this experiment, all of the toothbrushes had some type of microbial growth on them. Some level of

growth of all of the tested microorganisms was demonstrated, as shown in Table 1. There was microbial growth on 90% of the used toothbrushes. *Staphylococcus* sp. was the most common species in the samples, with 30% of the toothbrushes (n=20) presenting some growth of this organism. Twenty percent of the samples showed growth of *Lactobacillus* sp. *Streptococcus* sp. were found in 5% of the samples. Yeast cell sp. were found in 20% of the samples. Three samples developed *Pseudomonas fluorescens* equated to 15% of all the samples.

None of the samples yielded any fungal species nor did they yield *E. coli*.

The mean CFU per 1ml was 71.2/1ml for *Lactobacilli* sp. Yeast cell sp. had a mean CFU of 29.7/1 ml. *Staphylococcus* sp recorded a CFU of 8.2/1ml, *Streptococcus* sp had a record of 3/1 ml while *pseudomonas fluorescens* had a CFU of 6.7/1ml.

The percentage of bacteria isolated from toothbrushes stored in bathrooms was 65% more than that of brushes outside the bathroom. Brushes stored outside the bathroom had a contamination percentage of 25%.

Bacteria isolated from brushes stored inside bathrooms had *Lactobacilli* sp. colony counts that were larger (where the largest recorded colony was 97 CFU/ml) than that of the *Lactobacilli* sp. isolated from toothbrushes stored outside the bathroom (61 CFU/ml).

The three samples that were contaminated with *pseudomonas fluorescens* were stored outside the bathroom.

The brushes that were used twice a day were more contaminated than the brushes were used only once or those used three times.

The toothbrushes obtained from individuals who had used the brand Signal of toothpaste were found to have the highest bacterial counts.

The Kirby-Bauer test antibiotic sensitivity for all isolates was conducted against five antibiotics. Table 2 shows the results of the resistance testing towards different antibiotics.

The *pseudomonas fluorescens* was found to be resistant towards Ampicillin, Cefuroxime, Amoxicillin/clavulanate, Ceftriaxone, Cefepime. Seven isolates were sensitive towards Cefuroxime, Cefepime. Intermediate resistance was displayed by only two isolates.

Discussion:

It has been shown in the literature that toothbrushes are excellent locations for the growth of microorganisms. Toothbrushes are excessively contaminated by microorganisms during their everyday use and contaminated bristles may become an instrument of transmission and inoculation through gingival abrasions [5].

In this study showed the presence of different species of bacteria namely: *Staphylococcus* sp., *Lactobacilli* sp., *Yeast cell* sp. and *pseudomonas fluorescens* as well as *Streptococcus* sp.

The results of this study were very similar to those reported by other researchers [8,9]. Staphylococci were found in large numbers. Although they belong to the oral microbiota, *Staphylococcus* species deserves greater attention because it is capable of causing many oral infectious diseases [13,16]

The reinoculation of bacteria into the original host can pose a significant risk of dissemination for certain patients, such as immunosuppressed individuals, organ transplant recipients, and patients with cardiac conditions in whom transient bacteremia occurs after routine brushing with contaminated toothbrushes thus favoring the occurrence of bacterial endocarditis [10,20]. All species isolated can cause lower respiratory tract infections, skin and soft tissue infections, urinary tract infections, intra-abdominal infections, septic arthritis, osteomyelitis, ophthalmic infections.

Several previous studies have reported the isolation of *Lactobacilli* and *pseudomonas* from toothbrushes [1].

Yeasts were identified from 15% of brushes and *Streptococcus* was found in 10%. Such low numbers could possibly be due to the aerobic culture condition which somewhat inhibits their growth [17]. A particularly worrying trait of the isolates is their resistance to Ampicillin and other antibiotics. Infections of *Pseudomonas fluorescens* are an unusual cause of disease in humans, and usually affect patients with compromised immune systems.

In this study, toothbrushes that were kept in the bathroom environment recorded more isolates than those stored outside the bathroom after normal brushing. Also those toothbrushes that were used twice a day had the highest microbial colony counts.

In a survey of the Saudi population's oral health habits, 47% were found to store their brushes inside the bathroom and 35% reported brushing twice (unpublished work). Storage conditions of toothbrushes are an important factor for bacterial survival. The present investigation's results may be due to the fact that a wet environment increases bacterial growth and cross contamination [5,15].

In this study, the toothbrushes obtained from individuals who had used the brand "Signal 2" of toothpaste were found to have the highest bacterial counts. This may be slightly biased because a large percentage of the volunteers used "Signal 2" making it the most commonly used brand in the sample as well as the population (55% of the Saudi population reported using this brand; unpublished work).

Finally, it is worth noting that the findings of three samples contaminated with the unusual species of *Pseudomonas fluorescens* prompted the researchers to contact their owners for further details regarding their oral health. It was found that two of the samples came from two sisters, one of whom was a chronic asthma

patient who was intermittently on prolonged corticosteroid therapy. The two brushes were stored together in a single holder, thus explaining why both samples exhibited the growth. Further information about the oral health of the user of the third brush displaying *Pseudomonas fluorescens* could not be obtained.

Several studies recommend changing toothbrushes every three months; this is best when the individual is healthy. This is not what most health surveys report as over 48% of Saudis tend to keep using their brushes well over 3 months (unpublished work). However, sick children or adults should replace their toothbrushes as soon as possible to prevent reinfection or infection of another person. Population groups at risk, such as those that are immunocompromised, must also remain vigilant to the possibility of re-inoculation via toothbrushes and change them accordingly [10].

Finally, it is worth noting that the transfer of microorganism from one toothbrush to the other is quite possible through direct contact and therefore, should be considered when a family member is ill or susceptible to prevent infection spread.

Table 1: The average total numbers for each microorganism and the mean.

Bacteria species isolated	Positive toothbrush (# of sample/n)	CFU/1ml	Signal 2	Crest	Sensodyne	CFU inside bathrooms	CFU outside the bathroom
<i>Staphylococcus sp.</i>	30% (6/20)	8.2/1ML	3/20	1/20	-	8.2	7
<i>Lactobacilli sp.</i>	20% (4/20)	71.2/1MI	1/20	1/20	1/20	75	60
<i>Yeast cell sp.</i>	20% (4/20)	29.7/1 MI	1/20	1/20	1/20	29.75	--
<i>Streptococcus sp.</i>	5% (1/20)	3/1 ml	-	1/20	-	9	--
<i>Pseudomonas fluorescent</i>	15% (3/20)	6.7/1MI	4/20	-	-	--	6.66
No growth	10% (2/20)	-	2/20	-	--	--	--

Table 2: Antibiotic resistance of microbes isolated from samples.

Sample number	Bacteria species	Ampicillin	Cefuroxime	Amox_clavu	Ceftriaxone	Cefepime
2	<i>Staphylococcus sp.</i>	S	S	I	S	S
4	<i>Lactobacillus sp.</i>	R	S	S	S	S
7	<i>Lactobacillus sp.</i>	S	S	S	I	S
9	<i>Lactobacillus sp.</i>	S	S	S	S	S
10	<i>Staphylococcus sp.</i>	R	S	R	S	S
16	<i>Lactobacillus sp.</i>	S	S	R	S	S
18	<i>Staphylococcus sp.</i>	R	S	R	R	S
19	<i>Pseudomonas fluorescent</i>	R	R	R	R	R
20	<i>Pseudomonas fluorescent</i>	R	R	R	R	S

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