Original Article

Microbiological Study For Isolation Of Staphylococcus Aureus From Toothbrush

Hussein Sattar Abbood[1*], Noor Ridha Kadhim[2]

[1,2]Ministry of Education, Governorate of Baghdad, Iraq.

***Corresponding author:** Hussein Sattar Abbood

[1,2]Ministry of Education, Governorate of Baghdad, Iraq. email: Smurtadha28@gmil.com
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Abstract

The study was carried out by collecting 50 different samples, including 25 samples of used Toothbrush and 50 samples of unused Toothbrush, 16 isolates belonging to 64% staphylococcus aureus were isolated from samples taken from used Toothbrush while not isolated of staphylococcus aureus from Toothbrush was unused. Indicating that it is one of the leading causes of toothbrush contamination which later leads to inflammation of the mouth and teeth. Using biochemical tests, which included the catalase test, fermentation of mannitol sugar, and the API test, the isolates of staphylococcus aureus were 100% positive. The ability of bacteria to produce catalase enzyme is one of the most virulent agents of these bacteria, which leads to multiple infections in the mouth.

Keywords: Microbiological, Study, Isolation, Staphylococcus aureus, Toothbrush

1. INTRODUCTION

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

The Toothbrush is used daily to clean the oral cavity. A new toothbrush is usually not a favorable habitat for bacteria and fungi, but in some cases, toothbrushes are already slightly infected before use. Toothbrushes are shown to be contaminated in the oral cavity environment and from hands, aerosols, and the storage environments. The typical storage conditions of toothbrushes may act as a reservoir for reintroducing potential pathogens to the oral cavity and introducing other potential pathogens from the bathroom environment. These microorganisms have the potential to colonize the oral cavity due to the micro-trauma that tooth brushing can cause (Downes, 2008). Bacteria that attach to, accumulate, and survive on toothbrushes may be transmitted to the individual, causing disease. Several articles have reported the bacterial and fungal contamination of brushes, with higher or lower contamination associated with numerous interferences between the meeting and the handle. The Toothbrush has been characterized as a means of microbial transport, retention, and growth, and highly contaminated brushes may cause constant reinfection, a risk factor for periodontal disease (Efstratiou, 2007).

Toothbrushes play an essential role in oral hygiene and are generally found in community and hospital settings. They have been reported to be heavily contaminated with microorganisms when in regular use and may play a significant role in disease transmission and increase the risk of infection since they serve as a reservoir for microorganisms in healthy, oral-diseased, and medically-ill adults. Contaminated toothbrushes have been suggested to play a role in systemic and localized diseases. The possibility of toothbrushes being associated with the transmission of heart diseases, arthritis, bacteremia, and stroke has also been reported (Frazelle, 2012).

This study aimed to isolate, characterize, and identify staphylococcus, the bacterial contaminants by staphylococcus aureus on used manual toothbrushes.

2. LITERATURE REVIEW

The most important and most frequently applied tool for regular dental care is the Toothbrush. In this context, electric toothbrushes are more and more regularly used for the mechanical reduction of dental plaque because they show superior cleaning efficiency than traditional manual toothbrushes. Apparent differences in the mechanical efficiencies of the rotation/oscillation-based or the sonic-based technical approach have not yet been demonstrated (Tagi,1998). Toothbrushes are usually used for several weeks or even months, leading to colonization with the oral bacterial flora of the user, including facultative pathogenic bacteria. Continuous re-exposition by tooth brushing may maintain persisting

oral infections or lead to (auto-)reinfections. Accordingly, changing the Toothbrush at least once a month is advisable. Oral infections due to tooth brushing-associated lesions of the oral mucous membrane have been repeatedly described. Accordingly, there might be a minimal risk of disease due to tooth brushing, further minimizing the bacterial colonization on toothbrushes. (Wetzel, 2005**)**

Data on bacterial colonization of toothbrushes are scarce, particularly for electric toothbrush heads. Therefore, the effects of automated brushing and sonic-based toothbrushes on the bacterial colonization of the brush heads are unknown. In addition, it is further unclear whether easy-to-perform methods like rinsing, rinsing, and drying, application of disinfectants, or U.V. radiation after usage of manual and electric toothbrushes might effectively reduce their bacterial colonization (Caudry,1995).

The study assessed whether simple chlorhexidine disinfection or rinsing with tap water might reduce bacterial colonization on electric toothbrushes. Different types of toothbrushes were included in the analysis. Further, the decolonizing effects of U.V. toothbrush sanitizers on manual and sonic toothbrushes were assessed. UV-C radiation is suitable for reducing bacterial colonization on toothbrush heads. Finally, the impact of bristle thickness on the effects of decolonizing procedures was evaluated by comparing two manual toothbrushes. (Devine, 2007**).**

Toothbrushes are necessary for daily oral hygiene, but residues remaining on their bristles may precipitate the growth of several microorganisms. Over 700 bacterial species and fungi, viruses, and transient organisms are present in the oral cavity that may or may not cause various diseases. (Goldschmidt, 2004).

As early as 1920, Cobb reported the Toothbrush as a cause of repeated infections in the mouth. Many bacteria are found in toothbrushes after brushing, and the microorganisms maintain their viability, ranging from one day to one week. In addition, toothbrushes are frequently stored in the bathroom or close to the toilet and sink and may be exposed to enteric bacteria dispersed by aerosols. Even tiny droplets from the bathroom lead to the release of millions of bacteria into the atmosphere. The contamination mainly increases when toothbrushes are shared or stored together. Several factors, including the long survival time of the microorganisms, storage circumstances, and toothbrush location, cause the reintroduction of potential pathogens and cross-infection to the oral cavity (A. Osho, 2013).

Contaminated toothbrushes may play an essential role in many oral and systemic diseases, including septicemia and gastrointestinal, cardiovascular, respiratory, and renal problems. Some studies have suggested disinfecting toothbrushes to prevent various diseases using different methods. This condition is crucial for children, the elderly, and high-risk patients, including immunosuppressed individuals or those undergoing organ transplantation or chemotherapy. Although other methods have been investigated for toothbrush disinfection in the literature, this matter has received little attention from many researchers because most clinicians still consider toothbrushes only as caries and plaque-controlling devices (Malmberg, 1994).

Bacteria in the genus Staphylococcus are pathogens of man and other mammals. Traditionally they were divided into two groups based on their ability to clot blood plasma (the coagulase reaction). The coagulase-positive staphylococci constitute the most pathogenic species of S aureus. The coagulase-negative staphylococci (C.N.S.) are now known to comprise over 30 other species. Staphylococci can cause many forms of infection. S aureus causes superficial skin lesions (boils, styes) and localized abscesses in other sites. (Rasigade, 2014). S aureus causes deep-seated infections, such as osteomyelitis, endocarditis, and more severe skin infections (furunculosis). S aureus is a significant cause of hospital-acquired (nosocomial) infection of surgical wounds and, with Sepidermidis, causes diseases associated with indwelling medical devices. S aureus causes food poisoning by releasing enterotoxins into food. The S aureus causes toxic shock syndrome by releasing superantigens into the bloodstream. S saprophytic causes urinary tract infections, especially in girls. Other species of staphylococci (S lugdunensis, S haemolyticus, S warneri, S schleiferi, S intermedius) are infrequent pathogens. (Chambers, 2005).

Staphylococci are Gram-positive cocci 1μm in diameter. They form clumps. S aureus and S intermedius are coagulase positive. All other staphylococci are coagulase-negative. They are salt-tolerant and often hemolytic. Identification requires biotype analysis. S aureus colonizes the nasal passage and axillae. S epidermidis is a common human skin commensal. Different species of staphylococci are infrequent human commensals. Some are commensals of other animals. (Boucher, 2008)

Staphylococcus aureus expresses many potential virulence factors. Surface proteins that promote colonization of host tissues. Factors that probably inhibit phagocytosis (capsule, immunoglobulin binding protein A). Toxins that damage host tissues and cause disease symptoms. Coagulase-negative staphylococci usually are less virulent and express fewer virulence factors. S epidermidis readily colonizes implanted devices. Phagocytosis is the primary mechanism for combatting staphylococcal infection. Antibodies are produced which neutralize toxins and promote opsonization. The capsule and protein A may interfere with phagocytosis. Biofilm growth on implants is impervious to phagocytosis. (Tong SY, 2015).

MATERIAL AND METHODS

A- Equipment

Table (2-1): Equipment used in the study

origin	Equipment
Germany	slides
Germany	cover slides
Holanda	microscope
china	petri dish
Germany	incubator
china	syringe

B- Materials

Table (2-1): Materials used in the study

origin	material
Oxoid/ England	**gram stain**
Oxoid/ England	**nutrient agar and nutrient broth**
Oxoid/ England	**Mannitol Salt Agar**
Iraq	**distilled water**
Iraq	**H2O2**
Germany	**A strip**

1-Data Collection

Twenty-five Toothbrushes used from 1 to 60 days were collected from people aged 18-24, and 25 samples of Toothbrushes were not used (control) to investigate *staphylococcus aureus*. *S. aureus* isolates were diagnosed On microscopic and cultures characteristics and biochemical tests, According to (Forbes 2002).

2- Identification

1- **Culture Diagnosis:** The media of the first isolation is the nutrient agar to grow the samples in a dilution method and incubated at an optimal temperature of 37° C for 18-24 hours. (Tong, 2015).

- Preparation of nutrient agar according to the manufacturer's instructions and sterilization by autoclave at 121 ° C for 15 minutes.
- Distributing the nutrient agar that is prepared on Petri dishes.
- Samples are grown in the nutrient agar in a dilution method and incubated at an optimal temperature of 37 ° C for 18-24 hours.
- Note the results

2-Microscopic Diagnosis: Stain of bacteria growing on the nutrient agar by Gram stain to identify the type of bacteria. (Forbes, 2002).

- **Smear preparation:** A part of the colony is transferred to a clean, sterile slide with methanol and then stabilized by heat, leaving the solid to cool down before starting the staining

- Stain with Gram
- Immerse the heat-resistant Slide in crystal violet for 1 minute
- Wash the slides with water for 2 seconds
- Immerse the Slide with iodine for 1 minute
- Wash the slides with water for 2 seconds
- Immerse the Slide with alcohol (acetone) for 10-15 seconds
- Wash the Slide with water for 2 seconds
- Immerse the Slide in the opposite color (safranin) for one minute
- Wash the Slide with water for 2 seconds
- Note the staining results by microscopic at 100x.

3- Biochemical Test: using biochemical tests that include

1. Catalase test

Add a few drops of hydrogen peroxide to the concentration of 0.03 on the part of the colony transferred to the Slide (colony age 24 hours). The bubbles collected on the Slide's surface (*S. aureus*) will signify that the Test is positive. (Forbes, 2002).

2 - Mannitol Salt Agar test

Mannitol Salt Agar **was** prepared according to the manufacturer's instructions and sterilized by autoclave at 121 ° C for 15 minutes. (Forbes, 2002).

- The Mannitol Salt Agar is transported from the autoclave to cool at 50° C
- Distribution of the Mannitol Salt Agar on sterile Petri dishes in 15 ml each dish.
- Leave the dishes sterile for a period to harden
- After hardening the media, the samples are culture in a planning manner and incubated at an optimal temperature of 37 for 18-24 hours.
- Note the results

3- API test

The API RAPIDES staph system detects the production of an *S. aureus*-specific enzyme, aurease. Aurease undergoes a reaction with prothrombin, and the resulting product cleaves a fluorescent peptide in the Test well, thereby liberating fluorescence. This assay yields results of *S. aureus* or non-*S. Aureus*. The resulting pellet was added to 250 µl of demineralized water in the API strip and adjusted to match an internal 4.0 McFarland standard. Then 50 µl of the bacterial

suspension was added to control and Tested well on the strip. The strip was incubated at 35°C in non-CO2 for two h. The wells were examined for fluorescence using a Wood's lamp (365 nm). Although more fluorescence in the test well than in control well, positive results were subjectively determined. (van Griethuysen, 1998).

1- Statistical analysis

All the study results were subjected to statistical analysis to determine the differences of significance, and the T-test was used for this purpose. The differences were determined at the 5% probability level (Schielfer, 1980).

RESULTS AND DISCUSSION

1-Rate of isolation *Staphylococcus aureus* from Toothbrush

The study's main aim is to investigate the *Staphylococcus aureus*, which causes the contamination of the Toothbrush. The results showed that 16 isolates belonging to *Staphylococcus aureus* were obtained from samples taken from a used toothbrush. The results showed that 64% of the total showed bacterial growth. In comparison, those that did not show an increase in *Staphylococcus aureus* were nine samples 36%) of the total showed bacterial growth, while isolates belonging to *Staphylococcus aureus* were not obtained from control samples (of the capacity showed bacterial growth). The results of the statistical analysis showed significant differences in the number of the positive and negative models at the level of P <0.05, which indicates that *Staphylococcus aureus* is one of the most important causes of toothbrush infection, which causes the inflammation of the gums and teeth as in Figure (3-1). (Neal,2003)

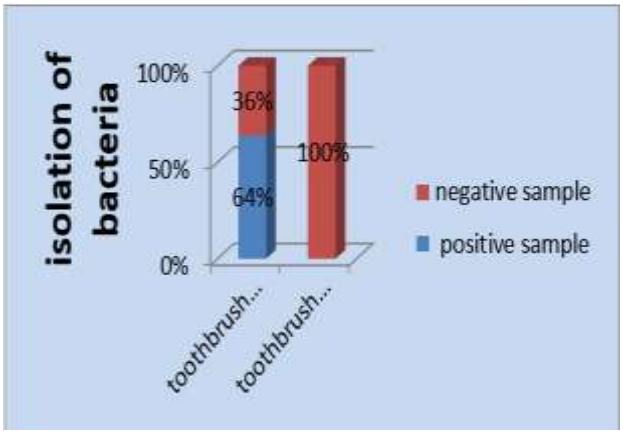

Figure (3-1): Rate of isolation *Staphylococcus aureus* from Toothbrush

P-value=0.001 statistical analysis
P-value < 0.05

2-Culture characteristic of *Staphylococcus aureus* in Toothbrush.

Staphylococcus aureus bacteria were diagnosed after they were cultivated and purified using the cultural characteristics of the colonies on the liquid and solid media. They were cultured on the nutrient broth and nutrient agar, the first isolation media. After an incubation period of 18-24 hours. At an optimal temperature of $37C^0$, large colonies (2-3 mm) are observed, dark, slightly convex, smooth edges, yellow-white or yellow-orange (golden). Figures (3-2) indicate the presence of staphylococcus bacteria. According to (Quirynen 2003; Sammons, 2004).

Figure (3-2): *Staphylococcus aureus* colonies on nutrient agar and broth.

3- Microscopic characteristic of *Staphylococcus aureus*

Microscopic properties were determined by microscopic examination of bacterial cell isolates, their arrangement, and gram dyes characteristics. For *Staphylococcus aureus,* as well as for (morphological tests), microscopic examination by

gram-stained for *Staphylococcus aureus* a contaminated toothbrush showing pairs or clusters figure (3-3), indicating the presence of *Staphylococcus aureus*, as according to (Smith, 2003).

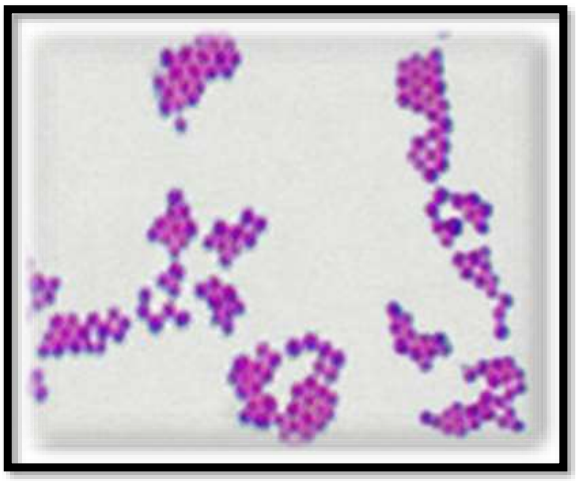

Figure (3-3): *Staphylococcus aureus* colonies staining with gram stain appear clusters shape.

4-Biochemical tests for isolation and diagnosis of *Staphylococcus aureus*

Biochemical tests were used according to the methods reported by (Benson 2002; Warren, 2001) To distinguish *S. aureus* bacteria from other bacterial species. The catalase test to determine *S. aureus* bacteria from the different types of staphylococcus *S. epidermidis* and *S. saprophyticus* differentiates between the various bacterial strains. It is the system of cytochrome enzymes found in aerobic bacteria (except the group of spores). The results showed that isolates of all bacteria were positive for the catalase test, as shown in Fig. (3-4). This indicates that the isolates produce the catalase enzyme by the extracellular *Staphylococcus aureus* and their production of this enzyme suggests that they are pathogenic strains. The catalase enzyme works according to the following equation:

$$2H_2O_2 + \frac{H^{\circ}}{H_{\circ}} \rightarrow 2H_2O + O_2 \uparrow$$

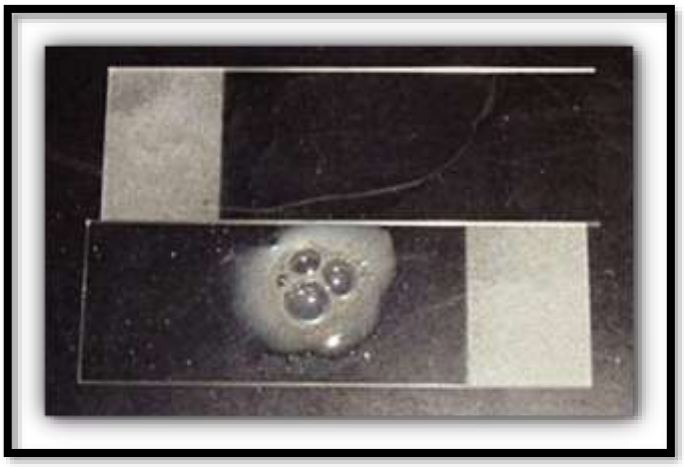

Figure (3-4): Staphylococcus aureus colonies appear positive results to catalase enzyme test

The Mantel fermentation test was used to distinguish *S. aureus* bacteria from *S. epidermidis*, which cannot ferment mannitol, often a small colony in a red or violet area. Furthermore, the Mantel fermentation test is also used to confirm the diagnosis of *Staphylococcus aureus* because it is capable of fermenting the mantel sugar. So, when Mannitol Salt Agar (containing 7.5% table salt, mantel, and red phenol) is added to a portion of the colonies, S. streptococcus aureus develops on nutrient agar. After 24 hours of incubation, the yellow colonies are surrounded by a yellow area, As in figure (3-5). indicating the fermentation of mannitol sugar, these results were identical (Alshayeb 2011).

Figure (3-5): Differentiate between *S. aureus* colonies Mantol fermentation and *S. epidermidis*, not fermentation

API Test

The API STAPH-IDENT system was compared with conventional methods to identify 14 *Staphylococcus* species. The API STAPH-IDENT strip utilizes a battery of 10 miniaturized biochemical tests. These tests include alkaline phosphatase, urease,-glucosidase, P-glucuronidase, Bgalactosidase activity, aerobic acid formation from D-(+)-mannose D-mannitol, D-(+)-trehalose, and salicin, and utilization of arginine. Reactions of cultures were determined after five h of incubation at 35°C. Results indicated a high degree of unity (>90%) between the expedient API system and conventional methods for most species. Figure (3-6). The results revealed that 64% of the total bacterial growth is *Staphylococcus aureus*, while those that did not show a *Staphylococcus aureus* were 36%) of the total showed bacterial growth. (MURRAY, 2003).

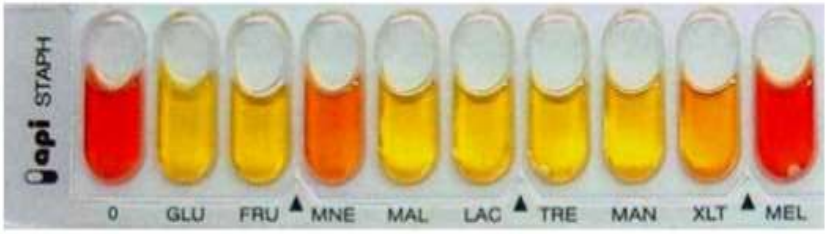

Figure (3-6): API staph combination of standard biochemical tests and fermentation tests which are the references tests for the identification of *staphylococcus aureus*

CONCLUSIONS AND DISCUSSION

This study was achieved by isolating *Staphylococcus aureus* with high rates of Toothbrush. This study also determined the percentage of *Staphylococcus aureus* in the Toothbrush. *Staphylococcus aureus* isolated from the Toothbrush can ferment mannitol sugar and the production of catalase enzyme, and these properties increase the ferocity of bacteria. A high percentage of *Staphylococcus aureus* from Toothbrush suffering from gingivitis aged 18-24 years. Recommend through this study the ongoing investigation of the presence of *Staphylococcus aureus* in the province so that this type of bacteria can be controlled and overcome the risks arising from infection. Therefore, it's essential to Conduct other studies on *Staphylococcus aureus* species, including determining other masculinity factors, such as its ability to produce enzymes Lipase, Hyalurinidase Collagenase, and Leukocidin, Deoxyribonuclase, and others. We need to use molecular choices in the diagnosis of Staphylococcus aureus by identifying their virulence genes.

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