



Microbiological Study For Isolation Of *Staphylococcus Aureus* From Toothbrush

Hussein Sattar Abbood

Ministry of Education, Governorate of Baghdad, Iraq.

*Corresponding author email: hussainss501@gmail.com

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

دراسة ميكروبيولوجية لعزل بكتيريا *Staphylococcus aureus* من فرشاة الأسنان

حسين سطار عبود

وزارة التربية، محافظة بغداد، العراق.

*البريد الإلكتروني للمؤلف hussainss501@gmail.com

خلاصة

أجريت الدراسة من خلال جمع 50 عينة مختلفة، منها 25 عينة من فرشاة الأسنان المستعملة و 50 عينة من فرشاة الأسنان غير المستخدمة، وتم عزل 16 عينة تنتمي إلى 64% من المكورات العنقودية الذهبية من العينات المأخوذة من فرشاة الأسنان المستعملة بينما لم يتم عزل المكورات العنقودية الذهبية من فرشاة الأسنان غير المستخدمة. مما يدل على أنه أحد الأسباب الرئيسية لتلوث فرشاة الأسنان والذي يؤدي فيما بعد إلى التهاب الفم والأسنان. وباستخدام الاختبارات البيوكيميائية والتي شملت اختبار الكاتلاز، وتخمر سكر المانيتول، واختبار API، كانت عزلات المكورات العنقودية الذهبية إيجابية بنسبة 100%. تعتبر قدرة البكتيريا على إنتاج إنزيم الكاتلاز من أكثر العوامل فتكاً بهذه البكتيريا، مما يؤدي إلى التهابات متعددة في الفم.

الكلمات المفتاحية: ميكروبيولوجي، دراسة، عزل، المكورات العنقودية الذهبية، فرشاة الأسنان

1. INTRODUCTION

The bacterial flowers in the human oral cavity is greater diverse than any different anatomical region. More than seven hundred species have been identified, four hundred of which have been located in periodontal pockets near teeth; organisms no longer normally related with oral flora have also been remoted from toothbrushes, along with Enterobacter, (Sammons et al.,2004). This enables contagious bacteria that were on the brush to spread to our oral teeth again. Some of these illnesses can even spread to other areas of our bodies, leading to major health issues including coronary heart disease, stroke, arthritis,



blood-borne illnesses like bacteremia and chronic illnesses, and many more. A toothbrush may serve as a breeding ground for billions of germs thanks to a variety of factors, including moisture from bathroom flushes and splashes. (Warren *et al.*, 2001).

Every day, mouthwash is used to clean the teeth. Although fresh toothbrushes are often no longer a good home for microorganisms and fungus, in particular circumstances, toothbrushes can quickly become infected before usage. It has been established that toothbrushes can get contaminated through contact with hands, aerosols, storage, and the oral environment. Normal toothbrush storage settings can act as a reservoir for the entrance of various controllable germs from the restroom environment as well as the return of manageable pathogens to the oral cavity. These bacteria have the potential to colonize the mouth since brushing can cause minor damage (Downes, 2008). Brushes may harbor, collect, and harbor bacteria that can transfer to people and cause illness. Brush bacterial and fungal infections have been discussed in several studies, and higher or lower disease has been linked to a number of interferences between affect and handling.. Toothbrushes are described as a car for the transport, retention and increase of microorganisms, and a heavily contaminated toothbrush can lead to persistent reinfection, a hazard component for periodontitis (Efstratiou, 2007).

Dental hygiene is very essential, and toothbrushes are frequently seen in public places and hospitals. As a microbial reservoir for people with health, dental, and clinical issues, they are reportedly highly contaminated with bacteria from routine usage and can play a crucial role in the spread of illness and increased infection risk. It has been suggested that contaminated toothbrushes may contribute to both systemic and localized illness. There have also been reports of tooth brushing being linked to the spread of heart disease, arthritis, bacteremia and stroke (Frazelle, 2012).

This study aimed to isolate, signify and identify *Staphylococcus aureus*, a bacterial illness precipitated by using *Staphylococcus aureus* on used guide toothbrushes.

2.LITERATURE REVIEW

The toothbrush is the most important and often used piece of equipment for routine dental care. Due to its greater cleaning efficacy compared to standard manual toothbrushes, electric powered toothbrushes are being employed in this context to mechanically reduce plaque. Technical techniques based on rotational/oscillation or sonic technology no longer show appreciable mechanical efficiency differences. (Tagi,1998). After using a toothbrush consistently for a few weeks or even months, the user's oral bacterial flora, which includes facultative pathogens, becomes colonized. Continued re-exposure through brushing can lead to persistent oral infections or lead to



(automatic) re-infections. Therefore, it is recommended to replace your toothbrush at least once a month. Oral infections caused by brushing-related lesions of the oral mucosa have been described repeatedly. Therefore, the risk of disease caused by brushing is likely to be minimal, further reducing bacterial colonization of the toothbrush. (Wetzel, 2005)

On the bacterial colonization of toothbrushes, particularly electric driven toothbrush heads, there is limited information available. Because of this, it is unknown how automated brushing and sonic toothbrushes affect the bacterial colonization of brush heads. Additionally, it is questionable if simple techniques like washing, rinsing, and drying, using disinfectants, or using UV radiation can be employed. With guide and electric toothbrushes, post-irradiation significantly lowers bacterial colonization. (Caudry, 1995).

The study looked at whether washing electric toothbrushes with tap water or using a basic disinfectant like chlorhexidine may lessen bacterial colonization. The research has covered a variety of toothbrush kinds. moreover, UV light's decolonizing impact. Both manual and sonic toothbrushes had been used for testing toothbrush sanitizers. It is possible to reduce bacterial colonization on toothbrush heads by using UV-C radiation. Finally, by comparing the performance of two manual toothbrushes, the impact of bristle thickness on the efficacy of the decolonization technique was assessed. (Devine, 2007).

A toothbrush is crucial for day by day oral hygiene, but the residue left on the bristles can accelerate the increase of a number microorganisms. There are more than seven hundred bacteria and fungi, viruses and transient organisms in the mouth that may or may also no longer motive a variety of illnesses (Goldschmidt, 2004).

As early as 1920, Cobb pronounced toothbrushes as a purpose of recurrent oral infections. Many micro organism are located in a toothbrush after brushing, and the microbes stay alive for somewhere from a day to a week. Additionally, toothbrushes are regularly positioned in lavatories or close to bogs and sinks and might also be uncovered to aerosol-borne intestine bacteria. Millions of germs can be released into the air by even the smallest drops in a restroom. When toothbrushes are used or kept together, contamination is very high. The reintroduction of potential pathogens into the oral cavity and cross-infection are caused by a number of variables, including long-lived microorganisms, storage conditions, and toothbrush placement. (A. Osho, 2013).

Contaminated toothbrushes may also be a significant factor in a variety of systemic and oral disorders, including sepsis and gastrointestinal, cardiovascular, pulmonary, and renal issues. Many studies recommend certain methods of sanitizing toothbrushes to prevent a range of infections. This



situation is critical for young people, the elderly, and high-risk patients, such as those who are immunocompromised or are undergoing chemotherapy or organ transplants. Although different toothbrush disinfection strategies have been reviewed in the literature, many researchers have paid little interest to this topic, as most dentists nevertheless view toothbrushes as simply a skill of controlling cavities and plaque (Malmberg, 1994).

Staphylococcus bacteria are pathogens that affect both humans and many creatures. Their conventional division into two businesses is solely based on their capacity to produce plasma clots (coagulase reaction). The most dangerous strain of Staphylococcus aureus is one that is coagulase-positive.. Coagulase-negative staphylococci (C.N.S.) are now acknowledged to consist of extra than 30 different species. Staphylococcus can reason many forms of infection. Staphylococcus aureus causes superficial pores and skin lesions (boils, styes) and localized abscesses someplace else. (Rasigade, 2014). Staphylococcus aureus causes deep infections such as osteomyelitis, endocarditis, and greater serious pores and skin infections (boils). Staphylococcus aureus is an essential cause of hospital-acquired (nosocomial) infections in surgical wounds and, together with pyoderma, reasons congenital clinical device-related disease. Staphylococcus aureus motives meals poisoning by means of releasing enterotoxins into food. Acid motives poisonous shock syndrome by using releasing superantigens into the blood. S saprophytic bacteria can cause urinary tract infections, in particular in girls. Other types of staphylococci (S. lugdunensis, S. haemolyticus, S. warneri, S. schleiferi, S. intermedius) are rare pathogens (Chambers, 2005). Staphylococci are Gram-positive cocci with a diameter of 1 μm . They shape lumps. Staphylococcus aureus and Staphylococcus intermedius were positive for coagulase. All other staphylococci have been bad for coagulase. They are salt tolerant and regularly hemolyzed. Identification requires biotype analysis. Staphylococcus aureus colonizes the nasal passages and armpits. Staphylococcus epidermidis is a frequent symbiont of human skin. Various staphylococci are rare human symbionts. Some are symbionts of different animals. (Boucher, 2008)

The virulence factors expressed by Staphylococcus aureus are many and useful. proteins on surfaces that encourage colonization of host tissues. Additional factors that might prevent phagocytosis (capsules, immunoglobulin-binding protein A). toxic substances that damage host tissues and cause disease symptoms. Staphylococci that lack coagulase are often less virulent and have fewer virulence factors. Implanted devices are easily colonized by S. epidermidis. The key defense against staphylococcal infection is phagocytosis. Produced are antibodies that aid in opsonization and neutralize poisons. Protein A and the capsule invade by phagocytosis. Biofilm growth on implants is phagocytosis-resistant. (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).

- Prepare nutrient agar according to manufacturer's directions and sterilize in an 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 heat-resistant glass slides in crystal violet for 1 minute
- Rinse slides with water for 2 seconds
- Soak slides in iodine for 1 minute
- Rinse slides with water for 2 seconds
- Soak slides in 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

Prepare mannitol salt agar in accordance to the manufacturer's instructions and sterilize by using autoclaving 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 Staphylococcus System detects the production of the Staphylococcus aureus-specific enzyme aurease. Aurease reacts with prothrombin, and the ensuing product cleaves the fluorescent peptide in the take a look at well, releasing fluorescence. The assay affords consequences from S. aureus or non-S. aureus. Staphylococcus aureus The resulting pellet was once added to 250 µl of demineralized water in an API strip and adjusted to meet the 4 McFarland inside standard. Then add 50 µl of the bacterial suspension to the manage and check properly on a take a look at strip. Incubate the strips in non-CO two at 35°C for 2 hours. Check the wells for fluorescence the use of a Wood lamp (365 nm). Although there is greater fluorescence in the check wells than in the manipulate wells, a high-quality result is subjectively noted. (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 primary objective of the study was to investigate Staphylococcus aureus, which causes toothbrush contamination. The results showed that 16 strains of *Staphylococcus aureus* were isolated from the used toothbrush samples.. 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 shows that Staphylococcus aureus is one of the most necessary causes of toothbrush infection, which causes the infection of the gums and enamel as in Figure (3-1).)Neal,2003)

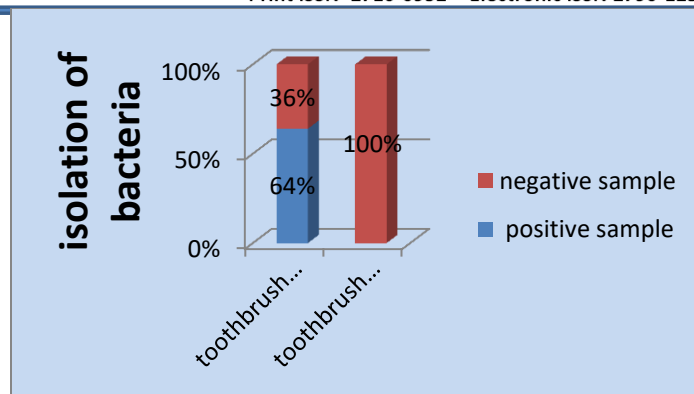


Figure (3-1): Rate of isolation *Staphylococcus aureus* from Toothbrush

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 37C⁰, 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 (Quiryneen 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).

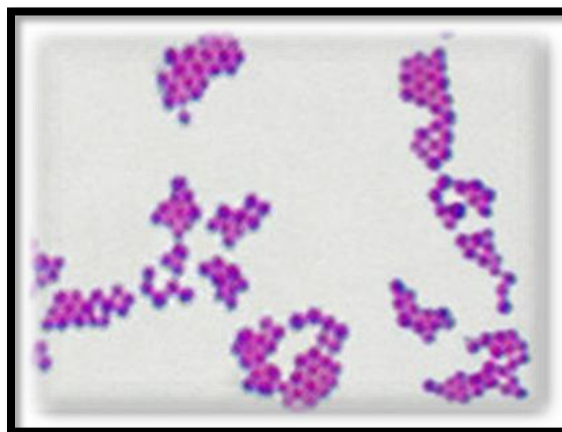


Figure (3-3): *Staphylococcus aureus* colonies staining with gram stain appear clusters shape.

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:

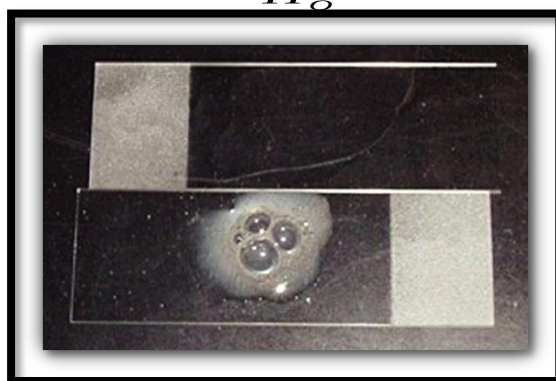
$$2H_2O_2 + \frac{H^O}{H^O} \rightarrow 2H_2O + O_2 \uparrow$$


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 gadget used to be compared with traditional techniques to perceive 14 staphylococci. The API STAPH-IDENT strip uses a set of 10 small biochemical tests. These assessments include alkaline phosphatase, urease, glucosidase, P-glucuronidase, beta-galactosidase activity, D-(+)-mannose, D-mannitol, D-(+)- Aerobic acidification of trehalose and salicin, and utilization of arginine. Incubation responses were measured after 5 hours of incubation at 35°C. Results show a high diploma of agreement (>90%) between the fabulous API device and conventional strategies 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 standard biochemical test and fermentation test combined, is the reference test 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, Hyaluronidase Collagenase, and Leukocidin, Deoxyribonuclease, and others. We need to use molecular choices in the diagnosis of *Staphylococcus aureus* by identifying their virulence genes.

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