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Design investigation about oral microbes causing dental caries of children under 12 years

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Abstract

This study is conducted to isolate and diagnose the aerobic bacteria species and fungi causing dental caries from children under twelve years who suffer from dental caries of both gender. During the period from 29 July to 9 December 2019, collecting fifty-nine specimens from patients who infected dental caries admitted to Al Afraz Health Center and outpatient clinics in the Samarra city. The result of isolation and diagnosis, seven bacterial types were shown in addition to the yeast like *Streptococcus mutans, Staphylococcus epidermidis, Staphylococcus aureus, Candida albucans, Escherichia coli, Pseudomonas aeruginosa, Klebsiella Sp, Proteus mirabilis*. Of the 59 patients with teeth decay, the *Streptococcus mutans* had scored the highest rate 26% of the total infections, followed by *Staphylococcus epidermidis* 22% while the lowest rate was scored by *Proteus mirabilis* 1%

Introduction

Dental caries is one of the most common chronic infectious diseases in the world [1,2] which involve initial demineralization of tooth followed by destruction of the organic phase of the dentine [3]. Tooth decay is chronic and progressing slowly over many years, and an important number of bacteria already present in the oral cavity cause these infections , and the body's immune system cannot determine these injuries and build immunity against them over time, and in the absence of appropriate treatment they develop leading to the tooth and surrounding tissues are damaged [4]. The mouth contains several types of bacteria, but a small number of them being caries, as the bacteria interfere with each other for adhesion and formation of biofilms, including (Oral *Streptococcus*) [5].

Tooth decay begins during a series of complex chemical reactions and as a result of the microbial activities associated with the formation of biofilms on the dental plaque, which leads to the removal of minerals from the calcified tissue of the teeth and the decomposition of organic components [6]. The carbohydrates in the diet are the main energy source under anaerobic conditions, which lead to the production of organic acids, which in turn reduce the pH to about pH 4.5, on the surface of the teeth, which causes the removal of minerals [7].

Therefore, the factor that causes decay is the production of acid and pH affected by the presence of bacteria and consume a lot of sugars [8,9], decay originates at first in a white spot without pain, and this spot develops and cavities appear with the appearance of pigments Brownish discoloration, and once the decay reaches dentine pain appears, due to thermal stimulation or because of eating sweets, food or sour drink [9,10]. The bacterial infection that leads to this disease is a chronic infection type that can continue and progress until the tooth is lost [11,12].

Although *Streptococcus* mutant is implicated as a major causative agent in dental caries, but that there are some people who have caries and do not have high levels of detectable levels of *S. mutans* where we found ratios of other bacterial isolates represented by *Staphylococus, Candida* and some types of Enterobacteriaceae*,* although *Candida* sp. is commonly found in the mouth, but it is usually an opportunistic pathogen [13,14]. The ability of *Streptococcus* to produce smooth surface decay is related to its ability to produce an adhesive matrix of sugars around bacterial cells. This matrix contributes to variety of properties related to decomposition, bacterial protection, and adhesion. For these reasons, *streptococcus* has specific carcinogenic characters and is considered the main factor in tooth decay [15].

Materials and methods

Sample collection and identification

The study includes collecting 59 samples from outpatient who suffer from dental caries and patients attending a dental clinic in the secretarial health centre in Samarra during the period from 29 July to 9 December 2019. Using sterile cotton swabs, samples were taken from the tooth under the direction and supervision of the specialized physician with filled in questionnaire forms. The questionnaire information included the names of the patients, age, place of residence, history of the disease, and the nature of the food intake.

Samples were diagnosed by culture in medium such as blood agar, MacConkey agar, mannitol salt agar, and incubated at 37°C for 24 hours. After incubation, the colonies are identified by colony morphology, lactose fermentation on the MacConkey agar, Gram stain and biochemical tests which included, coagulase, Oxidase, catalase, indole, methyl red, Voges-Proskauer, citrate utilization [6], motility (hanging drop), urease, in addition to Kligler iron agar, novobiocin test .

Results

This study included the collection of 59 samples of patients with tooth decay, as it isolated and diagnosed the types of bacterial. Fig. 1 shows the percentages of gram- positive microbes isolated from decay teeth. In this study showed the dominance of *Streptococcus mutans* that had the highest percentage was 26.31%, followed by *Staphylococcus epidermidis* 22.8 %, *Staphylococcus aureus* 19.29 %, and *Candida albicans* 14.03 %.

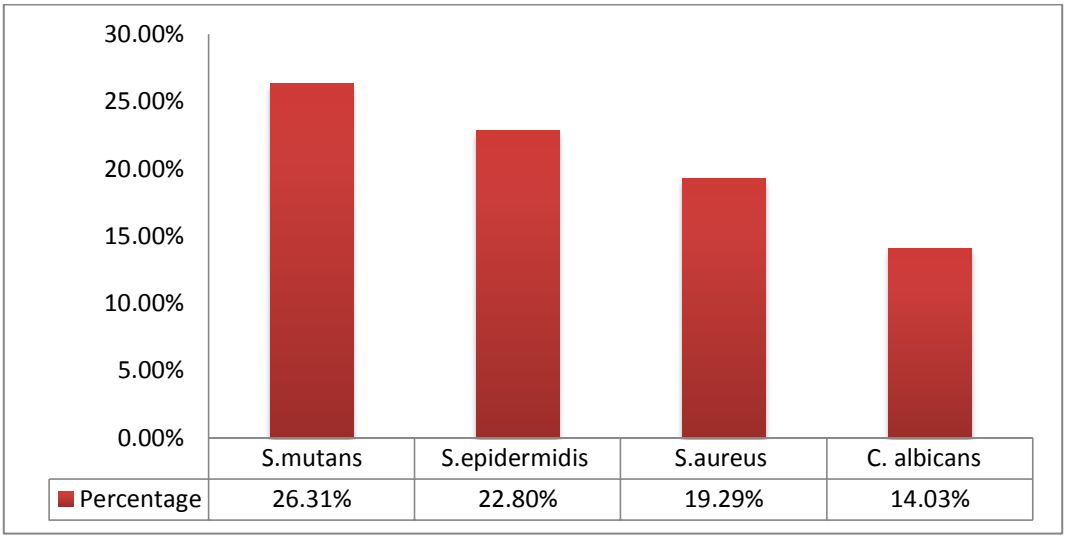

Fig. 1: The percentages of gram- positive microbes isolated from Dental caries

The results also show the presence of species belonging to the enterobacteriaceae, represented by *Escherichia coli* by 12.28 %, Fig. 2 shows the percentages, while *Pseudomonas aeruginosa* and *Klebsiella sp.* were 3.5 %, whereas the lowest rate was scored by *Proteus mirabilis* 1.7 % depending on microscopic exam, cultural characteristics and biochemical tests, such as the mannitol fermentation on the medium of mannitol as shown in Fig. 3(a), and novobiocin test was used as a test for sensitivity to novobiocin to differentiate between species of staphylococcus to differentiate between *Staphylococcus aureus* and *Staphylococcus epidermidis*, the results were recorded depending on the presence or absence of distinct inhibition regions, where *Staphylococcus epidermidis* is sensitive to novobiocin antibiotic as shown in Fig. 3(b).

Biochemical tests were used to differentiate between gram-negative bacteria such as catalase and oxidase test as shown in Fig. 3(c) and Fig. 3(d) and IMViC test (Indole test, Methyl-Red test, Voges- Proskauer test, and Citrate Utilization test). In Indole test, the presence of a reddish-pink ring in the alcohol layer is an indication of the positive test, as shown in Fig. 4(a). Methyl-Red test a red color is positive result, which an indication of the complete decomposition of glucose, as shown in Fig. 4(b), the change in the color of the medium from green to blue is considered evidence of positive test, as shown in Fig. 4(c), and urease test, the change in the color of the medium from yellow to pink red is evidence of the positive test, as the acidic function of the medium is changed to the base by the formed ammonia, as shown in Fig. 4(d). The 47 (80 %) samples has recovered gram-positive bacteria, while 12 (20 %) samples gram-negative bacterial. In this study, the rate of infections in females is higher comparing to that in males.

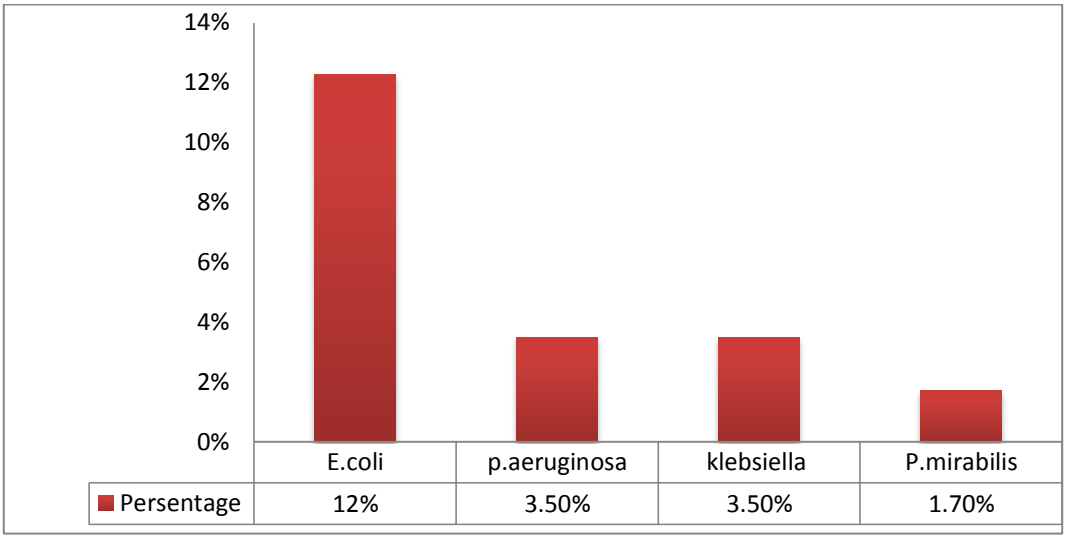

Fig. 2: The percentages of gram- negative bacteria isolated from dental caries

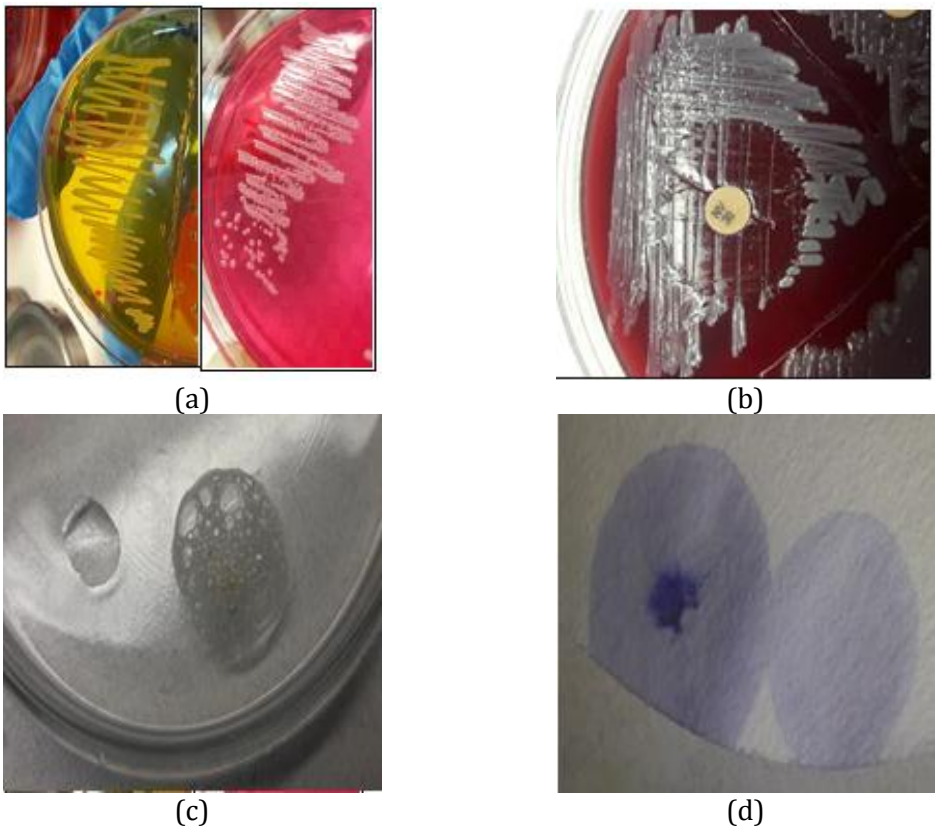

Fig. 3: (a) Mannitol salt agar, A mannitol fermentation for *staphyloccous aureus* and B non-fermentation of mantos for *Staphylococcus epidermidis*, (b) *Staphylococcus epidermidis* on blood agar with novobiocin disk, result sensitive, (c) Catalase test**,** a positive result is bubbles, a negative result is no bubbles**,** (d) Oxidase test purple color positive result, colorless negative result.

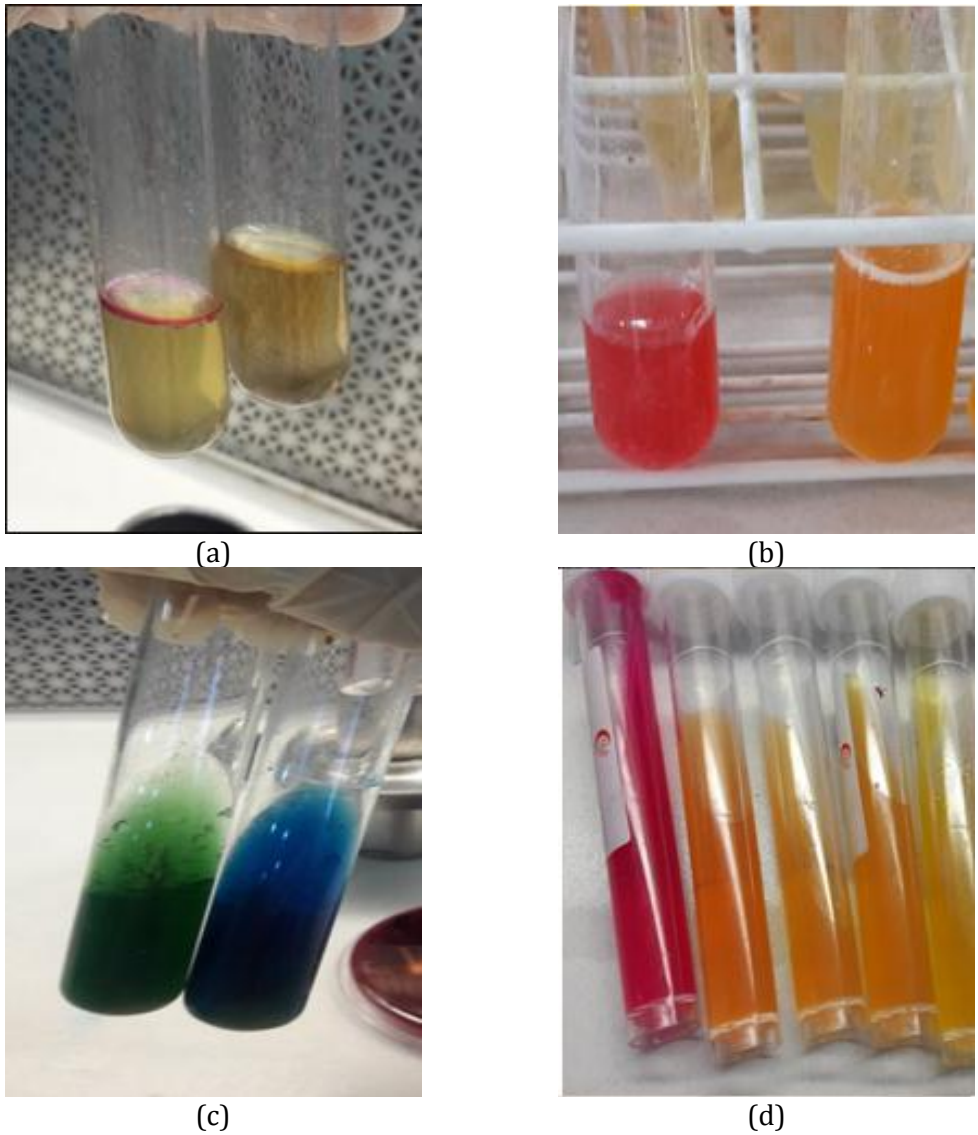

Fig. 4: (a) Indole test A: positive Result B: negative Result, (b) Methyl Red Test: red positive methyl red test, yellow negative methyl red test, (c) Simmon's citrate test: blue color positive result, green color negative result, (d) Urease test: pink color positive result, yellow negative result.

Discussion

In general, the results of this study are agree with what found by (Gibbons and Socransky) about the prevalence of *Streptococcus* in the mouth cavity. The reason for the difference in isolation rates may be due to the presence or absence of decay, as well as the number of decay teeth [16,17]. Explain Krass that the rates of isolation of the bacteria that cause mouth diseases differ according to the region of isolation and the type of infection, as well as the presence or absence of caries, So mention the number of bacteria *Streptococcus* and *Candida* it is present in a higher proportion in infected people than in healthy people. Its results confirmed that there was a difference in the isolation ratios according to the quality of the diagnosed sample [18].

The results of the study by Howell and his colleagues indicated that bacteria *Streptococcus* prevalent on the dental plaque. The percentage of isolation of microbes may be affected by the nature of the nutrients it deals with. Material handling also leads the container on the high

proportion of carbohydrates to the increasing rates of teeth decay and other oral problem, and this in turn leads to isolation ratios change, and believed that the quality of media user and isolation method and other unknown factors considerable influence in different isolation ratios, as well as the difference in the level of health awareness and continuous care in cleaning teeth and community culture, there is a great influence on the diversity of oral bacteria and teeth [19].

This small percentage of gram-negative bacteria comes from the scarcity of their presence in an environment, according to what many studies have confirmed, including a study by [20]. This is due to the fact that most gram negative bacteria come from infections of the respiratory system or gastro-intestinal tract and appear in the mouth, and that consistent with the findings of the study of [21,22]. This may be due to poor hygiene, the intromission of contaminated tools in the mouth, and playing with soil that leads to the pathogen entering from the external environment into the mouth. Bacteria enter also by water, food, air and hands.

References

1. Anusavice K. (2002). Dental caries: risk assessment and treatment solutions for an elderly population." Compendium of continuing education in dentistry., 23.(10): 12-20.

2. WHO .World Health Organization . (2002). The world health report 2002: reducing risks, promoting healthy life. World Health Organization.

3. Hamad A . (2OO8). A Clinical and Bacteiological of Oral and Dental diseases (dental caries) in Tikrit city." Tikrit Journal of Pure Science 13.(3): 125-127.

4. Cury et al. (2001). In situ study of sucrose exposure, mutans streptococci in dental plaque and dental caries." Brazilian dental journal . 12(2):101-104.

5. Kolenbrander et al. (2010). Oral multispecies biofilm development and the key role of cell–cell distance." Nature Reviews Microbiology 8.(7): 471-780.

6. Cardoso et al. (2011). Metabolic activity of Streptococcus mutans biofilms after treatment with different mouthwash formulations." Brazilian Journal of Oral Sciences 10.(1): 74-78.

7. Deng et al.(2007). The VicRK system of Streptococcus mutans responds to oxidative stress." Journal of dental research 86.(7) :606-610.

8. Zhang Song. (2013).Dental caries and vaccination strategy against the major cariogenic pathogen, Streptococcus mutans." Current pharmaceutical biotechnology 14.(11):960-966.

9. Amoroso P, Fernando A. and Célia M(2003). Prevalence of different Streptococci species in the oral cavity of children and adolescents." Brazilian Journal of Oral Sciences 2.(4): 164-168.

10. Makki M., and Mohammed Q . (2014). Study of some the pathogens isolated from the gums to the students of the Faculty of Science- ." International Journal of Advanced Research 2(10) :1007-1011.

11. Cross et al. (2007). Nanomechanical properties of glucans and associated cell-surface adhesion of Streptococcus mutans probed by atomic force microscopy under in situ conditions." Microbiology 153.(9): 3124-3132.

12. Ortner D . (2003). Identification of pathological conditions in human skeletal remains. Academic Press, 2nd Edition pp 645.

13. Amoroso P., Fernando A., and Célia M . (2003). Prevalence of different Streptococci species in the oral cavity of children and adolescents." Brazilian Journal of Oral Sciences., 2 (4):164-168.

14. Duailibe S., Alves C., Azizedite G., and Fernando J .(2007). Effect of a propolis extract on Streptococcus mutans counts in vivo." Journal of Applied Oral Science., 15.(5) :420-423.

15. Daly et al.(2001) . Bacteremia due to periodontal probing: a clinical and microbiological investigation." Journal of periodontology., 72.(2): 210-214.

16. Gibbons et al.(1964) . Studies of the predominant cultivable microbiota of dental plaque." Archives of oral biology., 9.(3): 365-370.

17. Socransky S., (1970).Relationship of bacteria to the Ethology of periodontal disease." Journal of dental research., 49.(2): 203-222.

18. Krasse B. (1954). The relationship between lactobacilli, Candida and streptococci and dental caries." Odontol. Rev., (5): 241-261.

19. Howell A., Rizzo A, and Paul F.(1965). Cultivable bacteria in developing and mature human dental calculus." Archives of oral biology 10.(2): 307-313.

20. Waltimo et al. (1997). Fungi in therapy-resistant apical periodontitis." International Endodontic Journal., 30.(2): 96-101.

21. Al-Khozai M. (2009). Inhibitory effects of Probiotic on growth and adhesion of some gram negative pathogenic bacteria." journal of kerbala university., 7.(1): 34-39.

22. الموسوي، علياء موسى.(2006). دراسة حول البكتريا الهوائية والخمائر المرافقة لبعض امراض الفم في مدينة الناصرية رسالة ماجستير، كلية التربية، جامعة ذي قار .

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التقصي عن الجراثيم الفموية المسببة لتسوس الاسنان للأطفال أقل 12 سنة

رواء حامد ايوب [1]*، هند عصام عبد الله [2]، سعادت مصطفى محمد [1]

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البحث مستل من رسالة ماجستير الباحث الاول

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الكلمات المفتاحية:

تسوس الأسنان، الميكروبات المسببة للأمراض، البكتيريا المعوية

الخلاصة:

أُجريت هذه الدراسة لعزل وتشخيص البكتريا الهوائية والفطريات من المرضى المصابين بتسوس الاسنان ومن كلا الجنسين من الاطفال دون سن الثانية عشر سنة خلال فترة من 29 تموز وحتى 9 كانون الاول 2019، لتحديد أنواع البكتريا الرئيسة التي قد يكون لها دور في تسوس الاسنان، تم جمع 59 عينة من مختلف الاطفال المصابين بتسوس الاسنان الوافدين الى مركز الافراز الصحي في مدينة سامراء وعيادات اطباء الاسنان الخارجية، وقد جمعت العزلات باستخدام ماسح قطني وشخصت بالاعتماد على الفحوصات المجهرية والاختبارات الكيموجيوية. أظهرت نتيجة العزل والتشخيص وجود سبعة انواع بكتيرية فضلاً عن الخمائر *Streptococcus mutans* , *Staphylococcus epidermidis* , *Staphylococcus aureus* , *Candida albucans* , *Escherichia coli* , *Pseudomonas aeruginosa* , *Klebsiella Sp* , *Proteus mirabilis* وجدت من بين 59 مريضا مصاباً بتسوس الاسنان بكتريا *S. mutans* بنسبة عالية بلغت 26 % من مجموع الجراثيم المعزولة الأخرى، وجاءت *staphylococcus epidermidis* بالمرتبة الثانية بنسبة 22 %، أما الاقل شيوعاً فهي بكتريا *Proteus mirabilis* بنسبة 1%.