



Investigation of some Gram-Negative Bacteria from Dental Caries in Diabetic Patients in Al-Muthana

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ABSTRACT

The study aims to determine the extent of the effect of diabetes on the spread of some Gram-negative bacteria that cause tooth decay by comparing their spread with those without diabetes (as a control group). The oral cavity is home to many different types of microbes (all of whom had dental caries under the age group of 13–70 years), to find out the extent of the effect of diabetes on increasing the frequency of these bacterial isolates. This study was conducted during the period from August 2023 to January 2024 in Samawah, in the Al-Muthanna Governorate. The specimens of tooth lesions were collected from a total of 100 diabetic patients and 50 non-diabetic patients under sterile conditions. Selective and sub culture media were used to culture and isolate the samples, and four bacterial species belonging to these strains were detected: *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Citrobacter Freundi*. Then bacteriological examination was performed. The frequency of bacterial isolates from the diabetic group was higher than that of non-diabetic patients, and their prevalence in women was higher than in males.

Keywords: Diabetes mellitus, dental caries, risk factors, Gram-negative rods.

INTRODUCTION

The oral cavity can serve as a reservoir for many pathogens of medical importance, including Gram-negative microorganisms, which present a wide range of virulence factors (Kumar, 2017). It has been confirmed that the presence of these bacterial species in the oral cavity is associated with various oral problems, such as gingivitis, tooth decay, and systemic diseases. Dental caries is a chronic, widespread disease caused by caries-causing bacteria attached to teeth that metabolize sugars to produce acid, which over time demineralizes the tooth structure (Pitts *et al.*, 2017). It can contribute to the cause of other systemic conditions if left untreated. The World Health Organization has declared that poor oral health may have an impact on health, mostly lifestyle, and many problems of the oral cavity are associated with chronic diseases such as diabetes mellitus (Wolf *et al.*, 2021). Diabetes mellitus is a common endocrine disorder, it is caused by a deficiency or ineffective production of insulin by the pancreas, which results in an increase or decrease in concentrations of glucose in the blood (Deshmukh *et al.*, 2015). Diabetes is classified into two types: Insulin-dependent diabetes mellitus (IDDM, type 1), an autoimmune disease characterized by an inflammatory reaction followed by selective destruction of insulin-secreting cells, and non-insulin-dependent diabetes mellitus (NIDDM, type 2), characterized by peripheral insulin resistance and impaired insulin secretion (Kumar *et al.*, 2020). This study aims to determine the prevalence of some Gram-negative bacteria from dental caries in diabetics patient from Al-Muthana province, and compare them with non-diabetic's patients.

MATERIALS AND METHODS

Sample Collection

The specimens of tooth lesions (dental caries) were collected from a total of 100 diabetic patients and 50 non-diabetic patients (they all had tooth decay) in Al-Muthanna province, for a period ranging from August 2023 until January 2024. Samples were collected at the Specialized Dental Center. Caries lesions were the sources of the samples.

Isolation and diagnosis of bacterial strains:

Sterile transfer medium was used and cultured directly on BHI broth medium to activate the samples, which were then incubated at 37°C for 48 hr. Tenfold serial dilutions were made. 0.1 ml of two dilutions (10^{-2} , 10^{-4}) were taken and then inoculated into the final growth medium and purified before being used in diagnostic tests, including MacConkey agar is used for the isolation of Gram-negative rod (GNR) bacteria, particularly members of the family Enterobacteriaceae, UTI Chromogenic Agar (for isolation *K. pneumoniae* and *E. coli*), Acetamide agar for isolation (*P. aeruginosa*), *E. coli*_coliform chromogenic agar (for isolation *E. coli* and *C. freundii*) as well as media enriched with 5% sheep blood were used. The growing colonies were isolated and purified, to perform cell morphological examination, Gram staining, and biochemical tests such as IMVIC test (Leboffe *et al.*, 2021). Bacterial isolates were diagnosed based on microscopic and biochemical characteristics such as colony culture characteristics (Al Jader *et al.*, 2022). Samples were taken based on the procedure adopted by (Zhou and Li, 2021), with some modification, as follows:

- The patient's mouth was rinsed with distilled water several times.
- The area of tooth decay and plaque is dried, then removed or scraped from the tooth by using a sterile probe.
- Swab transport media was used to take the sample from the probe then was transported to the laboratory.

Statistics analysis

The Social Statistical Package Science (SPSS) version 24 is used for statistical analysis. The chi-square test was used in this investigation. Rates are determined at a significance level of 0.05.

RESULTS AND DISCUSSION

The results of isolating samples from dental caries for both groups in 100 diabetic patients (males and females) aged between 13-70 years were Gram-negative bacteria. It belongs to the following strains: 43% of the isolates belonged to *K. pneumoniae*, 24% of the isolates belonged to *E. coli*, 7% of the isolates belong to *C. freundii*, while the lowest percentage was for *P. aeruginosa* isolates, where it was recorded at 4%. The frequency of bacterial isolates in general in the diabetic group was higher than in non-diabetic patients. As shown in Fig. (1).

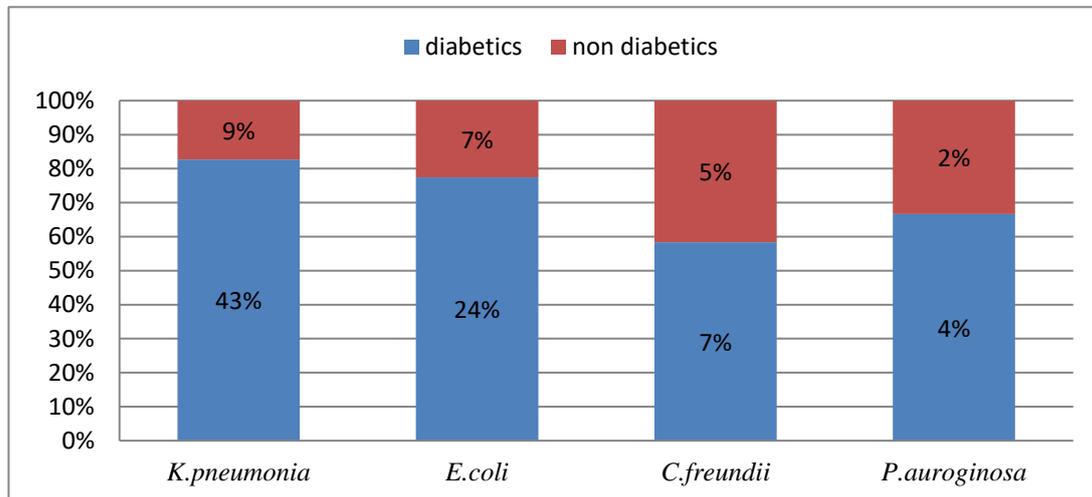


Fig. 1: Comparison between the frequency of bacterial isolates from dental caries in diabetic and non-diabetic groups.

Non-oral bacteria are non-resident microorganisms that are not usually viewed as members of the oral flora (Zaatout, 2021). Previous studies have found that non-oral bacteria, may occur in large numbers and transform from transient species to colonizers of the oral cavity in immunocompromised patients (Arirachakaran *et al.*, 2019). In diabetes, hyperglycemia causes a reduction in salivary flow, particularly with poor diabetes metabolic control. Saliva plays an important protective function in the oral cavity by cleaning food particles that feed tooth decay-causing bacteria. Saliva also removes acidic residue from food, neutralizing mouth acids that destroy tooth enamel. Remineralization is the process by which saliva restores the protective enamel of the teeth. Saliva contains calcium, phosphorus, fluoride and other minerals that strengthen the repair process and are resistant to tooth enamel surfaces. Therefore, the main reason for the increase in tooth decay may be dry mouth, which is one of the most important symptoms of diabetes (Fatima, 2020). During this time, glucose is present in the oral cavity, allowing the growth of bacteria as well as the development of tooth decay (Lati *et al.*, 2018).

Fig. (2) Shows the frequency of bacterial isolates according to sex (males and females) of diabetic patients. It was generally higher in females than in males.

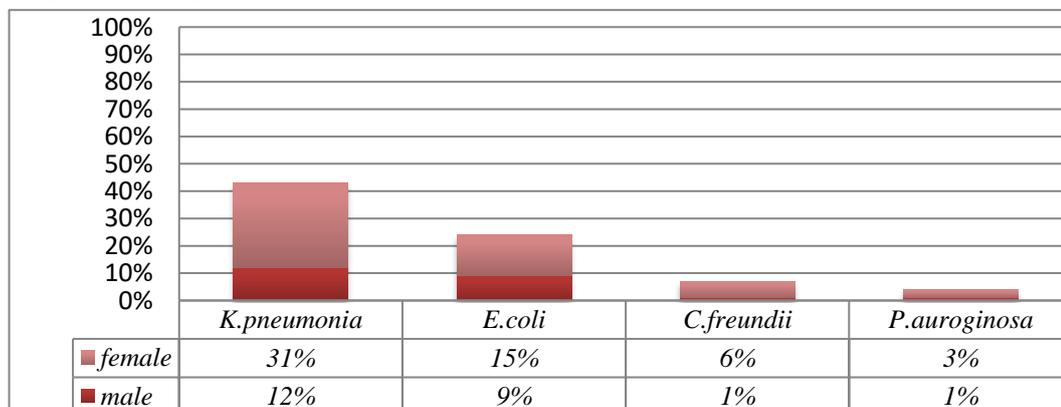


Fig. 2: A comparison between the frequency of some bacterial species of dental caries in diabetic patients for both sex males and females.

There may be a relationship between the early appearance of permanent teeth in the mouth, disparities in perceptions of oral health between the sexes, and a higher prevalence of tooth decay in women (Lipsky *et al.*, 2021). Enterobacteriaceae are important causes of serious infections, and many members of this family are becoming increasingly resistant to currently available antibiotics (de Oliveira *et al.*, 2015). Its presence in the oral cavity may be due to the use of drinking water, contaminated food, or poor personal hygiene (Zaatout, 2021). The frequency of Gram-negative bacteria in the oral environment is highly variable, and it has yet to be discovered if these bacteria are part of a colony or merely transient bacteria. However, they are usually associated with oral mucosal infections in immunocompromised patients. In these patients, infections of the oral mucosa may spread to other body systems and cause various health problems (Tada and hanada., 2010). Diagnosis is made on the basis of cultural characteristics of the isolated bacteria. *K. pneumoniae* colonies appeared on blood agar as non-hemolytic, mucous, and gray in color. On MacConkey agar, large, smooth, mucous, pink colonies appeared due to the presence of fermentable lactose in the medium. On UTI Chromogenic agar, the colonies were dark blue/purple in color, as shown in Fig. (3).

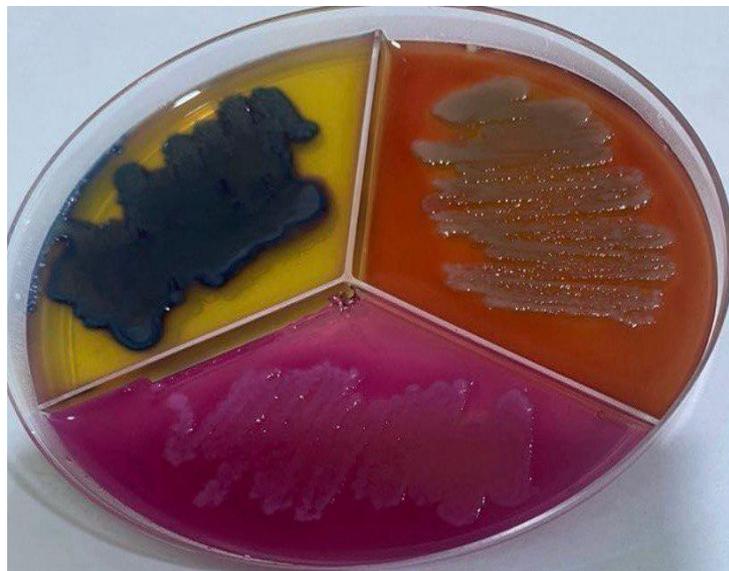


Fig. 3: *Klebsiella pneumoniae* on blood agar, MacConkey agar and UTI chromogenic agar.

In this study, the most prevalent genus in the diabetic group was *Klebsiella* which was found at a rate of 43%. Based on the results of this study, it was found that there is a significant difference between the frequency of bacterial isolates taken from dental caries in diabetic patients and isolates from non-diabetic patients, depending on the diabetes problems that affect the oral flora, and this is consistent with (Bisong *et al.*, 2014). Diabetes may cause poor functioning of the immune system, which may make it easier for bacteria to invade and spread the gums, thus exacerbating the infection. *Klebsiella spp.* can transform into various phenomena that are important for human diseases, such as colistin resistance, protection against oxidants and antibiotics because it uses biofilms containing a large number of bacteria that protect against antibiotics, especially in patients with weak immunity (Riquelme *et al.*, 2018).

P. aeruginosa colonies appeared on acetamide agar medium as small red-purple colours. While on MacConkey agar medium (used to distinguish bacteria capable of fermenting the sugar lactose from those that do not, especially members of the Enterobacteriaceae family), the colonies were pale in color because they had not fermented lactose, and the colonies also appeared mucoid, β -hemolytic on blood agar due to complete hemolysis as shown in Fig. (4).



Fig. 4: *Pseudomonas aeruginosa* on blood agar, MacConkey agar and Acetamide agar.

P.aeruginosa can be part of the transient oral microbiota but may colonize the oral cavity, perhaps due to its character as a strong aerobic bacterium (Arirachakaran *et al.*, 2019). Moreover, they have many virulence factors such as the ability to adhere and form biofilms on tissues and cellular surfaces (Tuon *et al.*, 2022).

E. coli on a MacConkey agar plate appear red/ pink in color due to their ability to ferment lactose and produce acids. While on UTI Chromogenic Agar medium, the colonies were pink or pale pink. As shown in Fig. (5).



Fig. 5: *E. coli* on MacConkey agar media and UTI Chromogenic agar.

The presence of *E. coli* is also considered a sign of oral and fecal contamination, so its presence in the oral cavity is considered as non-resident and transient microbes (Ladaycia *et al.*, 2021). Age-specific factors, for example immunological immaturity and lack of previous exposure to the most common infectious agents, children are especially vulnerable. Other factors that help the spread of bacteria include habits such as putting hands and objects in the mouth (Oliveira *et al.*, 2012). Whereas the colonies of *C. freundii* on MacConkey agar appeared as small, rough and dark pink (Lactose fermenter). On *E. coli* coliform chromogenic agar, the colonies had a salmon-to-red color due to the use of Salmon-Gal substrate in this medium as shown in Fig. (6).

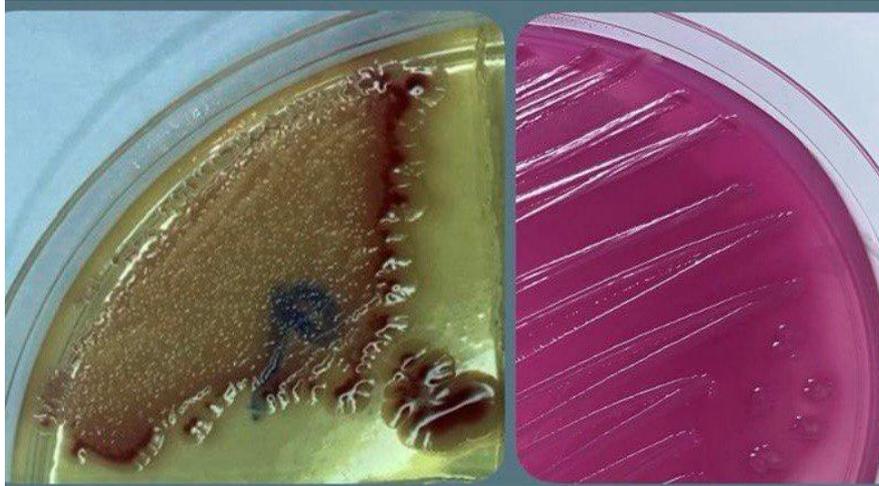


Fig. 6: *C. freundii* on MacConkey agar and *E. coli* coliform chromogenic agar media.

C. freundii is often considered a symbiont of the human intestinal flora. Some strains of this species have been reported and several virulence factors have been identified. It is often drug-resistant, possesses a capsule and produces toxins (Hess *et al.*, 2002). Our research findings are consistent with what has been documented (Sharma *et al.*, 2016), these are microorganisms from the *Enterobacteriaceae* family. They are opportunistic respiratory pathogens. Microscopic examination of the Gram stains revealed the presence of Gram-negative bacilli cells of the bacterial isolates, as shown in Fig. (7).

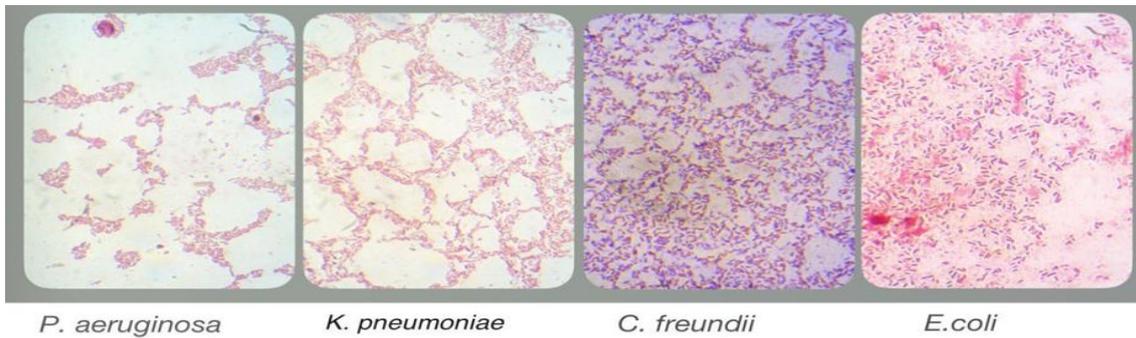


Fig. 7: The gram stain for bacterial isolate under microscope.

Positive oxidase test results occurred only in *P. aeruginosa*, and the violet color of the test appeared within 7-15 seconds on the filter paper. This indicates the ability of the bacteria to produce the enzyme oxidase. Bacterial species were also identified by biochemical tests, as shown in Fig. (8) which shows the results of biochemical tests on HIMVIC TM kit strips for: *E. coli*, *K. pneumoniae*, and *C. freundii*.



Fig. 8: Strips of HIMVIC TM Biochemical test kit for: A) *E. coli*. B) *K. pneumoniae*. C) *C. freundii*.

CONCLUSIONS

The present study showed that the frequency of bacterial species belonging to Gram-negative bacilli isolated from dental caries in diabetic patients is greater than that isolated from non-diabetic patients, which may contribute to the development of dental caries. This indicates the role of diabetes complications in exacerbating oral problems, especially caries. Therefore, more research using genome sequencing is needed to determine the role of these bacteria in the pathogenesis of oral cavity diseases.

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الكشف عن بعض البكتيريا سالبة الكرام من تسوس الاسنان لدى مرضى السكري في محافظة المثنى

فاديه صبحي ياسين

ميثم عباس مكي

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الملخص

يحتوي تجويف الفم على العديد من الميكروبات المختلفة هدفت الدراسة إلى الكشف عن مدى انتشار بعض البكتيريا سالبة الجرام المسببة لتسوس الأسنان لدى مرضى السكري وغير المصابين بالسكري (جميعهم أصيبوا بتسوس الأسنان تحت الفئة العمرية 13-70 عامًا) لمعرفة مدى تأثير مرض السكري على زيادة تواتر هذه العزلات البكتيرية. أجريت هذه الدراسة خلال الفترة من آب 2023 إلى كانون الثاني 2024 في مدينة السماوة في محافظة المثنى. تم جمع عينات آفات الأسنان من إجمالي 100 مريض مصاب بالسكري و50 مريضًا غير مصاب بالسكري تحت ظروف معقمة. تم استخدام أوساط الزراعة الانتقائية والفرعية لزراعة وعزل العينات، وتم اكتشاف أربعة أنواع بكتيرية تنتمي لهذه السلالات: *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Citrobacter freundii* ثم تم إجراء الكشف البكتريولوجي لها. وكانت نسبة تكرار العزلات البكتيرية من المجموعة المصابة بالسكري أعلى من تلك المعزولة من المرضى غير المصابين بالسكري، كما كان نسبة انتشارها في النساء أعلى منه في الذكور.

الكلمات الدالة: داء السكري، تسوس الأسنان، عوامل الخطر، العصيات سلبية الغرام.