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Molecular Diagnosis of *Enterobacter Cloacae* and *Stutzerimonas Stutzeri* and Study of Effect of Some Plant Extract

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Abstract

Periodontal pockets are thought to provide spawning grounds for a variety of microorganisms. Emergence of antibiotic-resistant bacterial strains poses a risk on course of treatment and may require surgical removal of gum pocket. This study's aim was to determine the bacteria found in gum pockets using PCR for 16S rRNA gene. As well as impact of plant extracts on growth of these strains. Five new bacterial strains have been registered at NCBI, including *Stutzerimonas stutzeri*, *Staphylococcus aureus*, and *Enterobacter cloacae*. All identified strains were susceptible to Augmentin and Ciprofloxacin but resistant to Cephalothin. Cefixime and Bacitracin were not effective against 90% of strains. Amoxicillin resistance rate was 50%. All strains were resistant to Turmeric and Oregano extracts at concentrations of 100 and 200 mg/ml, with exception of *Staph. aureus* FKZEW99, which was susceptible to Oregano at 200 mg/ml. Identified strains' responses to clove extract differed in terms of sensitivity and resistance. The overuse of antibiotics leads to appearance of new bacterial strains resistant to a range of treatments.

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التشخيص الجزيئي لبكتريا *Enterobacter cloacae* و *Stutzerimonas stutzeri* ودراسة تأثير بعض المستخلصات النباتية

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جامعة الموصل، كلية العلوم، قسم علوم حياة، الموصل، العراق.

المستخلص

يعتقد ان جيوب اللثة توفر بيئة مناسبة لتكاثر مجموعة متنوعة من الكائنات الحية الدقيقة. يكمن الخطر في ظهور سلالات جديدة من البكتريا مقاومة للمضادات الحيوية، مما يؤدي الى ابطاء عملية العلاج او الاستئصال الجراحي لجيب اللثة. الغرض من هذه الدراسة هو تحديد البكتريا الموجودة في جيوب اللثة باستخدام تقنية PCR لجين 16S rRNA.بالإضافة الى اختبار الحساسية للبكتريا المكتشفة، وكذلك تأثير المستخلصات النباتية على نمو هذه السلالات. تم تسجيل خمس سلالات بكتيرية جديدة في، NCBIبما في ذلك *Stutzerimonas stutzeri* و *Staphylococcus aureus* و *Enterobacter cloacae*. كانت جميع السلالات المشخصة حساسة Augmentin Ciprofloxacin ولكنها مقاومة Cephalothin. لم يظهر Cefixime Bacitraci فعالية ضد 90% من السلالات. في حين كان معدل مقاومة Amoxicillin 50%. أظهرت جميع السلالات مقاومة لمستخلصات الكركم والاوريجانو بتراكيز 100و200 ملغم /مل، باستثناء *S. aureus FKZEW99*، الذي كان عرضة للاوريجانو عند 200 ملغم/مل. اختلفت استجابات السلالات المشخصة لمستخلص القرنفل من حيث الحساسية والمقاومة. يؤدي الافراط في استخدام المضادات الحيوية الى ظهور سلالات بكتيرية جديدة مقاومة لمجموعة من العلاج.

الكلمات المفتاحية: المكورات العنقودية الذهبية، *Enterobacter cloacae*، اللثة، S rRNA16، المضادات الحيوية.

1. Introduction

The majority of periodontal disease pathogens are anaerobic bacteria as a result of biofilm development, which can also include facultative aerobic bacteria. Most bacterial species found in the periodontal pocket environment (Actinomycetes, certain Streptococci, and Staphylococci) can cause opportunistic infections in the afflicted regions. There is evidence that some intestinal bacteria, viruses, and Saccharomyces spp. may be present in periodontal pockets, indicating further infection caused by gingival damage. Periodontal disorders are now regarded as mixed illnesses caused by a variety of bacteria (Mombelli, 2018). *Tannerella forsythia*, *Porphyromonas gingivalis* and *Treponema denticola* are the main bacterial pathogens that cause gingivitis and advance the disease. Because of their common presence in gum pockets where gum tissue destruction is noticeable, these bacteria are referred to as the "red complex" (Drisko and Lewis, 1996; Giannopoulou *et al*., 2012).

Gingival tissue loss is one of the symptoms of periodontal disease, which results in alveolar bone loss and pocket development. To prevent and cure this condition, it is critical to understand the pathogenic function of the periodontal pocket's bacterial population. Scaling and root planning is a procedure for removing bacterial deposits under the periodontal by exfoliating the tooth and pocket surface, has been found in clinical tests to reduce pocket depth (Cappuyns *et al*., 2012; Popova *et al*., 2013).

The effectiveness of antimicrobial medications that are readily available locally, antiseptic washes, and systemic antibiotics has been assessed in several experiments. According to recent research, photodynamic treatment can be used to trigger the antibacterial activity of certain compounds in periodontal pockets (Suzuki *et al*., 2013; Haque *et al*., 2022).

The level of bacterial resistance to antibiotics varies by country, and this is due to differences in antibiotic usage. Although pathogenic bacteria are becoming more resistant to antibiotics, treating periodontal disease with antibiotics should still be done with caution (Kulik *et al*., 2008).

The current study sought to identify and diagnose several types of bacteria found in periodontal pockets, as well as determine the strains to which they belong. Furthermore, susceptibility testing was performed and plant extracts were used to examine their effect on the isolated bacteria.

2. Materials and Methods

2.1 Sample collection

The current study was conducted at the health center (Al –Arabi St.) on eight people suffering from periodontal pockets. The pocket was removed and cleaned using antiseptic cotton swabs. Each specimen was numbered and patient information was taken for future use as a guide. After that, the specimens were quickly sent to the laboratory for bacterial detection.

2.2 Isolation types of bacteria

To distinguish between Gram-negative and Gram-positive bacteria from each swab, the MacConkey-agar was used as a selective medium. The samples incubated for 24-48 h. at 37°C. The first diagnosis of the samples was made using morphological and phenotypic traits.

2.3 DNA extraction

Extracting genomic DNA straight from the samples using the Geneaid kit (Taiwan). We took the steps that the manufacturer recommended. Following measurements of its concentration and purity, genomic DNA was kept at -20°C for using in the next test.

2.4 Detection 16S rRNA gene using PCR

The PCR test kit from Promega (USA) was utilized. For the PCR experiment, a 20-µl GoTaq G2 Green Master Mix was produced. The complete the universal primers (27F) were utilized to amplify the 16S rRNA gene region. AGAGTTTGATCMTGGCTCAG and 1522R AAGGAGGTGATCCARCCGCA (Abdulrazzaq and Faisal, 2022). The manufacturer's instructions were followed when adding 100 ng of total template DNA and 1 µM primer concentration. The following was the setup for the 16S rRNA gene PCR protocol: Amplification in thirty cycles: thirty seconds of denaturation at 95°C, Annealing for 30 seconds at

55°C and extension for 1 minute at 72°C. Three minutes were spent at 95 °C for the first denaturation step. The last step of expansion was chosen at 72 °C. The molecular weight marker utilized was a 100 bps DNA marker obtained from New England Biolabs, UK (Abdulrazzaq and Faisal, 2022)**.**

2.5 DNA sequencing and alignment

PCR results from subsequent purification, the 16S rRNA gene was forwarded to Psomagene USA-Based Sequencing Company for nucleotide sequencing. Using NCBI's BLAST software, retrieved sequences were compared to previously known genes deposited to GenBank.

2.6 Antibiotic sensitivity test

The Kirby Bauer approach was used to conduct a sensitivity test. Three to five bacterial colonies were isolated from the agar plates and cultured in test tubes containing five milliliters of sterile saline. The uniformity of the bacterial cultures in the saline test tubes is next checked by comparing them to 0.5 MacFarland's standard. Each bacterial culture was aseptically swabbed onto the Muller Hinton agar surface (cross streaking method) using sterile swabs and left to dry. To ensure aseptic contact with the agar, the antibiotic discs (Augmentin, Ciprofloxacin, Cefixime, Amoxicillin, Bacitracin, Cephalothin) were gently pushed onto the plate's surface making use of sterile forceps. For 24 hours, the plates were incubated at 37°C. The millimeter-sized zone of inhibition was measured and monitored.

2.7 Preparation of plant alcoholic extracts

To make the alcoholic extracts, 20 g of the previously prepared botanicals (cloves, oregano, and turmeric) were immersed in 200 mL of ethanol 70% in the ice bath. The mixture was then refrigerated for 24 h, and the extraction and drying steps were performed at 40°C. The resulting powder was stored in sterilized opaque tubes at -20°C until used (Al-saidy *et al*., 2013).

2.8 Preparation of stock solutions alcoholic extracts

Each extract's powder was dissolved to the proper weight in 5 ml of DMSO (Dimethyl sulfoxide) to create stock solutions. Each stock solution received 200

mg/mL of extracts of clove, oregano, and turmeric. To maintain sterility, stock solutions were filtered using membrane filters featuring 0.45 µm holes (Al-Noamy, 2020).

3. Results

We have identified several different bacterial species based on the 16S rRNA detection types (gram positive and gram negative). We have identified five new strains. These unique strains were registered in the Gene Bank of NCBI with the accession numbers shown below:

Stutzerimonas stutzeri accession no.: FZE83 (OR616632), ZKEWF80 (OR616633)

Enterobacter cloacae accession no.: ZEF94 (OR616630), EFZ23 (OR616631)

Staphylococcus aureus accession no.: FKZEW99 (OR616634)

Figure (1) depicts the gel electrophoresis of the 16S rRNA gene, which permitted 5 clones for the newly registered strains. The DNA band size is 1495 base pairs.

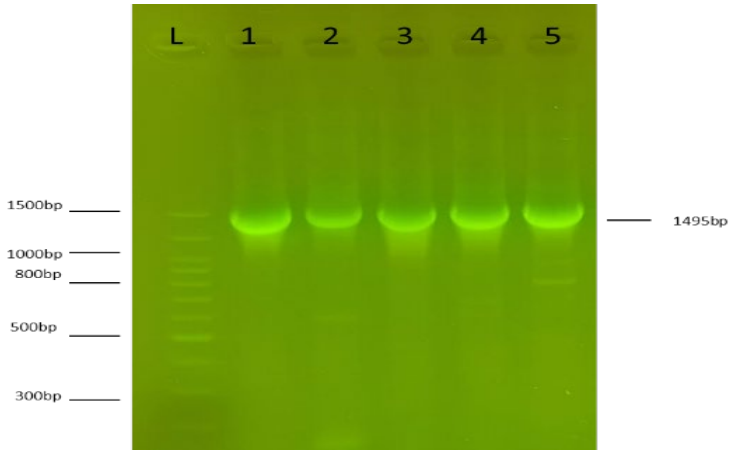

Fig 1: Gel electrophoresis of 16S rRNA.

3.1 Phylogenetic tree for the novel strains

Each novel strain was sequenced and aligned to determine the similarity with previously registered kinds. The Nucleotide Basic Local Alignment look Tool (BLAST) tool was used to look for homology between the input sequences and complete sequences available in the NCBI: GenBank database. Phylogenetic

trees were created using MEGA-11 software and a bootstrap analysis (Tamura *et al*., 2021).

S. stutzeri FZE83 and ZKEWF80 have 99% similarity to *S. stutzeri* ATCC tas17588, as shown in Figure (2).

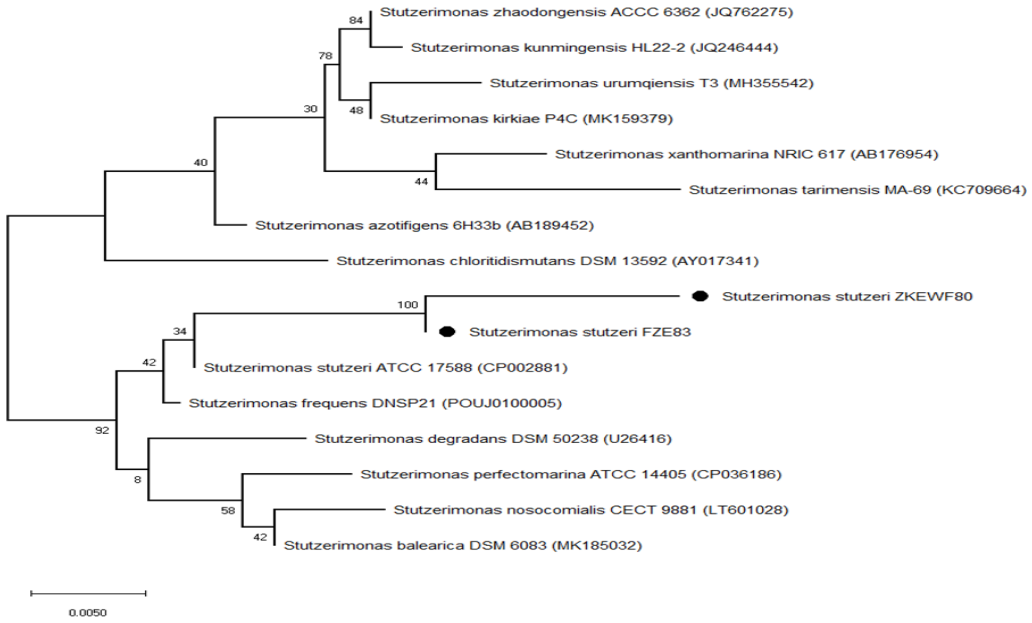

Fig 2: Neighbor-Joining phylogenetic trees depicting the connection between the strains *S. stutzeri* FZE83 and *S. stutzeri* ZKEWF80 (shown in black circles) and related Stutzerimonas spp. based on the gene sequences at a scale length of 0.005. The proportion of duplicate trees with related strains grouped in the bootstrap analysis (100 repetitions) is displayed next to each branch.

E. cloacae ZEF94 and EFZ23 exhibit a 98.74% similarity to *E. cloacae* ATCC 13047, as shown in Figure (3).

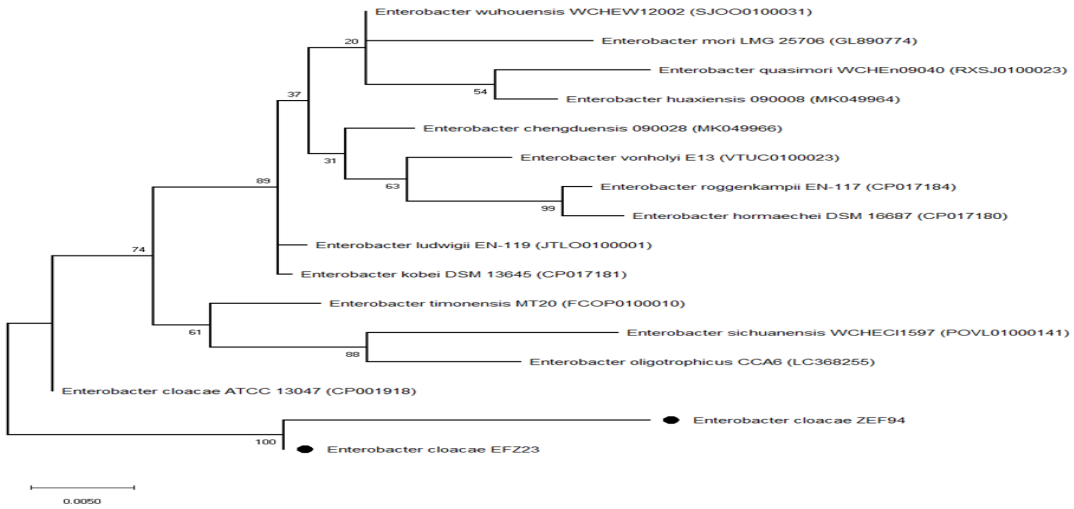

Fig 3 Neighbor-Joining phylogenetic trees demonstrating the connection between the bacteria *E. cloacae* EFZ23 and *E. cloacae* ZEF94 (shown in black circles) and the related *Enterobacter* spp. The proportion of replicate trees where the connected strains grouped collectively throughout the bootstrap experiment (100 repetitions) is displayed next to the branches.

Staph. aureus FKZEW99 shares 100% similarity with *S. aureus* DSM 20231, 99.1% with *S. simiae* NCTC 13838, and 98.57% with *S. epidermidis* NCTC 11047, as shown in Figure (4).

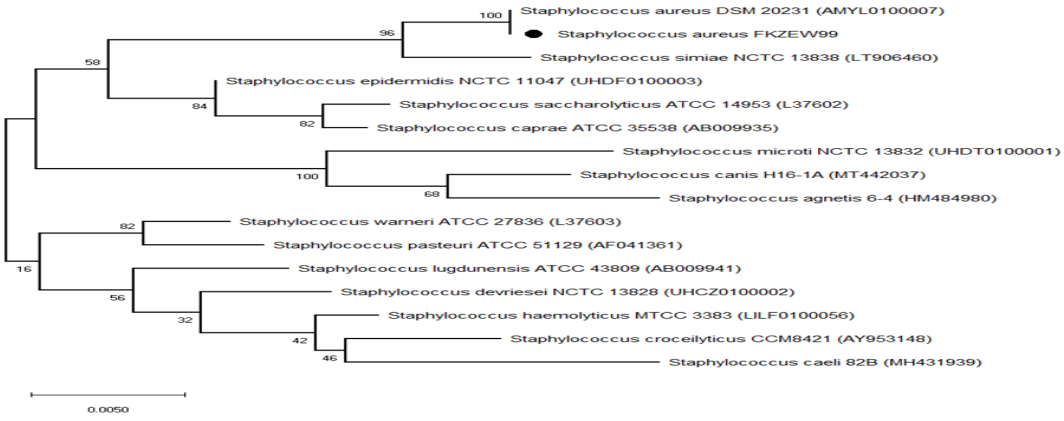

Fig 4: Neighbor-Joining phylogenetic trees depicting the connection between the strain *S. aureus* FKZEW99 (shown in black circle) and related *Staphylococcus*

spp. The proportion of duplicate trees in which the related strains grouped together in the bootstrap test (100 repetitions) is indicated next to the branches.

3.2 Antibiotic sensitivity test analysis

To select the greatest contagious microbes, we employed a diffusion of antibiotic disks technique, employing various antibiotics from distinct classes that vary based on their mechanism of action in opposition to the tested bacterium. Based on prior research, the diameter of bacterial growth suppression was measured to be 8 mm in the current study, depending on its sensitivity or resistance (Hassan *et al*., 2022). All newly recorded strains were shown to be responsive to the medicines Augmentin and Ciprofloxacin, while all were resistant to Cephalothin. However, all strains were shown to be 90% resistant to Cefixime and Bacitracin. Amoxicillin resistance rate was 50%, Table (1).

Table- 1- Antibiotic sensitivity test of the detected strains of bacteria.

Antibiotic	Interpretation					
	S. stutzeri FZE83	*S. stutzeri* ZKEWF80	*S. aureus* FKZEW99	*E. cloacae* ZEF94	*E. cloacae* EFZ23	%
Augmentin	S	S	S	S	S	100
Ciprofloxacin	S	S	S	S	S	100
Cefixime	R	R	R	R	I	90
Bacitracin	R	R	I	R	R	90
Amoxicillin	I	S	S	R	R	50
Cephalothin	R	R	R	R	R	100
%	60	50	40	50	60	

R = Resistant; S = Sensitive; I = Intermediate

3.3 Sensitivity test for plant extraction

Extracts of plants under study were used to observe the effect of these extracts on bacterial sensitivity or resistance compared to antibiotic susceptibility testing. The sensitivity test was performed in the same manner as measuring the diameter of bacterial inhibition in growth dishes. The diameter of inhibition was assessed on an 8 mm field in accordance with prior research (Montagna *et al*., 2019).

Table (2) illustrates the proportion of bacteria resistance and sensitivity to plant extract solutions at 200 mg/ml concentrations. Oregano has a 100% resistance rate, but turmeric has a 90% resistance rate, suggesting that both extracts are bacterially susceptible. Clove demonstrated a sensitivity of 70%, which is regarded good in terms of inhibitory activity. *S. stutzeri* FZE83 and ZKEWF80 strains were resistant to other plant extracts. This study found that the inhibitory findings for plant extracts at doses of 100 and 200 mg/ml were similar, with a little difference. All strains recorded demonstrated 100% sensitivity, except the *S. stutzeri* ZKEWF80 strain, which showed resistance to all extracts, Table (3).

Table-2- The impact of plant extracts on the development of new bacterial isolates when the extract is concentrated at 200 mg/ml.

	S. stutzeri FZE83	*S. stutzeri* ZKEWF80	*S. aureus* FKZEW99	*E. cloacae* ZEF94	*E. cloacae* EFZ23	%
Turmeric 200 mg/ml	R	R	I	R	R	90
Oregano 200 mg/ml	R	R	R	R	R	100
Clove 200 mg/ml	I	R	S	S	S	70
%	83.33	100	50	66.7	65	

Table -3- The impact of plant extracts on the development of new bacterial isolates when the extract is concentrated at 100 mg/ml.

	S. stutzeri FZE83	*S. stutzeri* ZKEWF80	*S. aureus* FKZEW99	*E. cloacae* ZEF94	*E. cloacae* EFZ23	%
Turmeric 100 mg/ml	R	R	R	R	R	100
Oregano 100 mg/ml	R	R	S	R	R	100
Clove 100 mg/ml	S	R	S	S	S	80
%	66.7	100	33.3	66.7	66.7	

4. Discussion

It is essential to research and identify the oral bacteria because of their importance to a person's health. A somewhat objective view of the species and composition of bacteria can be obtained from the evolution of the DNA sequence of the conserved bacterial 16S rRNA gene. This is useful for detecting bacteria that are highly sensitive, not cultivable, or strains that have just been discovered (Han *et al*., 2016).

Genomic sequencing of 16S rRNA can provide more information than standard approaches like qPCR or PCR. 16S rRNA gene sequencing has many advantages, which include identifying more bacteria in test samples, and giving information on dental pockets and the plaque microbiota (Shi *et al*., 2018).

The current investigation detected five novel strains of bacterial species found in periodontal pockets: *S. stutzeri, E. cloacae*, and *S. aureus*. These species are often found naturally in areas contaminated with a variety of bacteria and fungus, such as the mouth, gum tissues, and oral cavity. Although these strains have less mutations than strains recorded in NCBI, it is feasible to evaluate the influence of mutations on antibiotic resistance. The development of hitherto

unknown bacterium strains may raise alarms about their antibiotic resistance, increasing the complexity of treatment.

In one documented study, 42.7% of patients had *Staphylococcus* spp. in the periodontal pocket and 69.5% in the oral cavity. The study also found that S. aureus was 13.4% frequent in the gingival pocket and 15.8% in the oral cavity (Cuesta *et al*., 2010). Penicillin-resistant staphylococci are commonly found in subgingival biofilms, regardless of periodontal condition. Strains with virulence genes associated with inflammation, cytotoxicity, and tissue invasion contribute to these opportunists' pathogenicity in the gingival environment (Colombo *et al*., 2023).

Enterobacter spp. is important because it may make patients' clinical presentations of chronic periodontitis more complex. They are essential in situations where periodontitis is refractory, and they have the potential to cause systemic infections if they persist after periodontal treatment. There is a greater prevalence of *Enterobacter* in people with persistent gingivitis (Gamboa *et al*., 2013).

An interesting aspect of this study is the difference in antibiotic sensitivity and resistance among the bacterial strains studied, since all were resistant to Cephalothin but responsive to Augmentin and Ciprofloxacin. The sensitivity of the remaining antibiotics varies depending on the kind.

A previous study found that the susceptibility test for an isolated *S. stutzeri* Most of the tested antibiotics were effective against the strain from high-salinity wastewater, including Ciprofloxacin, Gentamycin, and Tobramycin (Wang *et al*., 2023). Previous research indicated that *E. cloacae* develop a chromosome-mediated AmpC β-lactamase, rendering them resistant to Ampicillin, Amoxicillin-Clavulanate (Augmentin), and first and second-generation cephalosporins. This study does not agree with the findings of our present study (Hu *et al*., 2020).

Excessive and inadvertent antibiotic usage promotes the spread of resistance among microorganisms. This is due to the phenomena of natural selection, which causes bacteria to adapt to the changing conditions in their

surroundings. If the dose of the antibiotic is not deadly to the bacteria, it causes adaptations, including mutations in the bacterium, which can lead to the bacteria gaining resistance through several methods (Bottery *et al*., 2022).

The present study's findings corroborated a previous investigation that found *E. cloacae* bacteria to possess innate resistance against both Ciprofloxacin and amoxicillin. Contrary to what we observed in our present work, this study discovered that *E. cloacae* are resistant to Augmentin. The previous study also discovered that the incidence of *E. cloacae* increased by 20% between 2008 and 2015, with antibiotic resistance increasing among periodontitis patients (Jepsen *et al*., 2022).

Regarding plant extract solutions' impact on bacterial proliferation, our investigation discovered that all registered strains are resistant to Turmeric and Oregano at doses of 100 and 200 mg/ml, respectively. One exception is the *S. aureus* FKZEW99 strain, which was sensitive to Oregano at 200 mg/ml and Turmeric at 100 mg/ml. The strains differed in their sensitivity and resistance to clove extract at the two observed concentrations.

Our results contrast those of a prior study that demonstrated that turmeric leaf extract at concentrations of 20%, 40%, 60%, and 80% reduced the development of *E. coli*, Staph. aureus, and *S. dysenteriae* but not *L. acidophilus* (Ilham *et al*., 2018). A laboratory study was done utilizing the agar diffusion method with the concentration of turmeric extract. The inhibitory zone was seen at 20%, 40%, 80%, and 100%. Turmeric extract inhibits the growth of *Staph. aureus* bacteria. These results are congruent with what we discovered in this investigation (Peterson *et al*., 2018).

Curcumin users increased their beneficial microbiota by an average of 69% in the identified species. The turmeric plant was used to treat intestinal disorders, and the microorganisms in the gut responded somewhat. The bacteria susceptible to it were *Clostridium spp., Bacteroides, Citrobacter, Enterobacter spp., Enterobacteriaceae, and Klebsiella* spp. (Fatimah *et al*., 2022).

The impact of oregano oil on biofilm-grown strains of *Staph. aureus* and *Staph. epidermidis* was assessed in a study. It was proven that the biofilm

inhibitory concentrations reduced the development of biofilms for *Staph. aureus* on polystyrene microtiter plates (Nostro *et al*., 2007).

Oregano essential oil is gaining popularity among academics due to its inherent antibacterial properties. Some studies have used proteomics to evaluate its possible antibacterial benefits against *Staph. Aureus* (Hao *et al*., 2021).

Although there have been few studies on cloves' antibacterial activity against *Enterobacter spp., Citrobacter spp., and Proteus spp*., one study discovered that cloves efficiently treated all of these clinical isolates. They demonstrated an antibacterial capability against several intestinal bacteria strains, which was quite comparable to our findings (Faujdar *et al*., 2020). Clove oil was tested for its bactericidal properties against *Staph. aureus* and *E. coli* O157:H7, and it was shown to reduce bacterial numbers by less than 3.0 logarithmic units (Yoo *et al*., 2021).

5. Conclusions

It is critical to diagnose bacteria in periodontal pockets since they are vital to an individual's health and can prevent future difficulties. The indiscriminate the usage of antibiotics causes the creation of novel strains of bacteria that are resistant to a variety of therapies. Not only that, but the newly identified strains some of these bacteria are resistant to plant extracts, the effectiveness of other natural products should be investigated

Conflict of interest

Regarding this study, according to the authors, there are no conflicts of interest.

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References

Abdulrazzaq, R., & Faisal, R. (2022). Efficiency of hichrome Enterococcus faecium agar in the isolation of Enterococcus spp. and other associated bacterial genera from water. Journal of Life and Bio Sciences Research, 3(01), 01-06.

Al-Noamy, N. A. (2020). Detection of the inhibitory effect of the leaves, seed and fruits of Cydonia oblonga on some gram positive and negative bacteria. Rafidain J. Sci, 29, 10-19.

Al-saidy, H. A., Al-qrtani, Y. M., & Al-Zubaidy, N. A. (2013). Effect of Crued Extracts for Cinnamum (Cinnamum zeylaicum), Syzygium (Syzygium aromaticum) and Thymus (Thymus vulgaris) on Growth of Aspergillus flavus which produce Aflatoxin B1. Diyala Agricultural Sciences Journal, 5(2), 593-602.

Bottery, M. J., Matthews, J. L., Wood, A. J., Johansen, H. K., Pitchford, J. W., & Friman, V. P. (2022). Inter-species interactions alter antibiotic efficacy in bacterial communities. The ISME journal, 16(3), 812-821.

Cappuyns, I., Cionca, N., Wick, P., Giannopoulou, C., & Mombelli, A. (2012). Treatment of residual pockets with photodynamic therapy, diode laser, or deep scaling. A randomized, split-mouth controlled clinical trial. Lasers in medical science, 27, 979-986.

Colombo, A. P. V., do Souto, R. M., Araújo, L. L., Espíndola, L. C. P., Hartenbach, F. A. R., Magalhães, C. B., ... & da Silva-Boghossian, C. M. (2023). Antimicrobial resistance and virulence of subgingival staphylococci isolated from periodontal health and diseases. Scientific Reports, 13(1), 11613.

Cuesta, A. I., Jewtuchowicz, V., Brusca, M. I., Nastri, M. L., & Rosa, A. C. (2010). Prevalence of Staphylococcus spp and Candida spp in the oral cavity and periodontal pockets of periodontal disease patients. Acta Odontológica Latinoamericana, 23(1), 20-26.

Drisko, C. H., & Lewis, L. H. (1996). Ultrasonic instruments and antimicrobial agents in supportive periodontal treatment and retreatment of recurrent or refractory periodontitis. Periodontology 2000, 12(1), 90-115.

Fatimah, B., Kinasih, D. S. S., Retnowati, W., & Setiawati, Y. (2022). Antibacterial Activity Test of Turmeric Extract (Curcuma longa) From

Madura Island Against Staphylococcus aureus. Jurnal Kesehatan Prima, 16(1), 74-82.

Faujdar, S. S., Bisht, D., & Sharma, A. (2020). Antibacterial activity of Syzygium aromaticum (clove) against uropathogens producing ESBL, MBL, and AmpC beta-lactamase: Are we close to getting a new antibacterial agent?. Journal of family medicine and primary care, 9(1), 180-186.

Gamboa, F., García, D. A., Acosta, A., Mizrahi, D., Paz, A., Martínez, D., ... & Abba, M. (2013). Presence and antimicrobial profile of gram-negative facultative anaerobe rods in patients with chronic periodontitis and gingivitis. Acta Odontológica Latinoamericana, 26(1), 24-30.

Giannopoulou, C., Cappuyns, I., Cancela, J., Cionca, N., & Mombelli, A. (2012). Effect of photodynamic therapy, diode laser, and deep scaling on cytokine and acute-phase protein levels in gingival crevicular fluid of residual periodontal pockets. Journal of periodontology, 83(8), 1018-1027.

Han, J., Wang, P., & Ge, S. (2016). The microbial community shifts of subgingival plaque in patients with generalized aggressive periodontitis following non-surgical periodontal therapy: a pilot study. Oncotarget, 8(6), 10609.

Hao, Y., Li, J., & Shi, L. (2021). A carvacrol-rich essential oil extracted from oregano (Origanum vulgare “Hot & Spicy”) exerts potent antibacterial effects against Staphylococcus aureus. Frontiers in Microbiology, 12, 741861.

Haque, M. M., Yerex, K., Kelekis-Cholakis, A., & Duan, K. (2022). Advances in novel therapeutic approaches for periodontal diseases. BMC oral health, 22(1), 492.

Hassan, M. A., Abd El-Aziz, S., Elbadry, H. M., Samy, A., & Tamer, T. M. (2022). Prevalence, antimicrobial resistance profile, and characterization of multi-drug resistant bacteria from various infected wounds in North Egypt. Saudi Journal of Biological Sciences, 29(4), 2978-2988.

Hu, K., Zeng, L., Zhang, J., Li, H., Su, S., Zhao, Y., ... & Zhang, X. (2020). Antibiotic susceptibility and molecular analyses of clinical Enterobacter cloacae isolates in Eastern Heilongjiang Province, China. Annals of Palliative Medicine, 9(3), 1211219-1211219.

Ilham, L. A., Herla, R., Dwi, S., & DewiRestuana, S. (2018). Antimicrobial activity of turmeric leaf extract against Escherichia coli, Staphylococcus

aureus, Shigella dysenteriae, and Lactobacillus acidophilus. In IOP Conference Series: Earth and Environmental Science (Vol. 205, No. 1, p. 012048). IOP Publishing.

Jepsen, K., Falk, W., Brune, F., Cosgarea, R., Fimmers, R., Bekeredjian-Ding, I., & Jepsen, S. (2022). Prevalence and antibiotic susceptibility trends of selected enterobacteriaceae, enterococci, and candida albicans in the subgingival microbiota of German periodontitis patients: a retrospective surveillance study. Antibiotics, 11(3), 385.

Kulik, E. M., Lenkeit, K., Chenaux, S., & Meyer, J. (2008). Antimicrobial susceptibility of periodontopathogenic bacteria. Journal of antimicrobial chemotherapy, 61(5), 1087-1091.

Mombelli, A. (2018). Microbial colonization of the periodontal pocket and its significance for periodontal therapy. Periodontology 2000, 76(1), 85-96.

Montagna, M. T., Triggiano, F., Barbuti, G., Bartolomeo, N., De Giglio, O., Diella, G., ... & Caggiano, G. (2019). Study on the in vitro activity of five disinfectants against nosocomial bacteria. International journal of environmental research and public health, 16(11), 1895.

Nostro, A., Roccaro, A. S., Bisignano, G., Marino, A., Cannatelli, M. A., Pizzimenti, F. C., ... & Blanco, A. R. (2007). Effects of oregano, carvacrol and thymol on Staphylococcus aureus and Staphylococcus epidermidis biofilms. Journal of medical microbiology, 56(4), 519-523.

Peterson, C. T., Vaughn, A. R., Sharma, V., Chopra, D., Mills, P. J., Peterson, S. N., & Sivamani, R. K. (2018). Effects of turmeric and curcumin dietary supplementation on human gut microbiota: A double-blind, randomized, placebo-controlled pilot study.

Popova, C., Dosseva-Panova, V., & Panov, V. (2013). Microbiology of periodontal diseases. A review. Biotechnology & Biotechnological Equipment, 27(3), 3754-3759.

Shi, M., Wei, Y., Hu, W., Nie, Y., Wu, X., & Lu, R. (2018). The subgingival microbiome of periodontal pockets with different probing depths in chronic and aggressive periodontitis: a pilot study. Frontiers in cellular and infection microbiology, 8, 124.

Suzuki, N., Yoneda, M., & Hirofuji, T. (2013). Mixed red-complex bacterial infection in periodontitis. International journal of dentistry, 2013(1), 587279.

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: molecular evolutionary genetics analysis version 11. Molecular biology and evolution, 38(7), 3022-3027.

Wang, Z., Sun, X., Chen, X., Wang, H., & He, H. (2023). The Mass Spectrometry Identification, Antimicrobial Genes Detection, and Proteomics Analysis of Stutzerimonas stutzeri Strain Was Isolated from Industrial Wastewater. Separations, 10(9), 461.

Yoo, J. H., Baek, K. H., Heo, Y. S., Yong, H. I., & Jo, C. (2021). Synergistic bactericidal effect of clove oil and encapsulated atmospheric pressure plasma against Escherichia coli O157: H7 and Staphylococcus aureus and its mechanism of action. Food Microbiology, 93, 103611.