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Ethanol Plant Extracts and Essential Oils Susceptibility and Molecular Characterization of *Facklamia hominis*: An Emerging Atypical Gram-Positive Pathogen

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ABSTRACT

This study aimed to detect the presence of atypical Gram-positive bacteria and evaluate their susceptibility to selected plant extracts and essential oils through analysis of 200 diverse clinical specimens collected from patients in many hospitals in Mosul/ Iraq. Specimens included blood, pus, vaginal swabs and urine, bacterial isolation and identification were performed using standardized microbiological techniques. Among the results, the species of the bacteria were very diverse, including Gram-positive and Gram-negative with particular distributions gained according to the source of a sample. Out of the isolates, 37 were Gram positive and they included general species of *Staphylococcus* spp.; *Streptococcus* spp.; *Enterococcus* spp.; and *Lactobacillus* Spp.

Gram negative included *Escherichia coli*, *Proteus* spp.; *Klebsiella* spp. and *Pseudomonas* spp. as well as limited fungal isolates mainly *Candida* spp. Some of the samples showed absence of microbial growth. Of these four rare strains of the genus *Facklamia* spp.; namely *Facklamia hominis*, only four times occurring in two urines and two vaginal swab samples and were confirmed by molecular investigation as species-specific PCR (*16SrRNA* gene sequencing). It was the first case of clinical strains of *F. hominis* to be reported in Iraq. These were deposited in GenBank (accession no. PV719694.1 to PV719697.1).

The comparative genomic results revealed considerable similarity to international strains, with local mutations, which might be the case of adaptation. Plant extracts and essential oils susceptibility testing provided different degrees of resistance which point to genetic diversity and plasticity of *F. hominis*. This amounts to clinical significance on the rise of *F. hominis* and the need of the molecular diagnostics to detect the rare and emerging pathogens.

Keywords: *Facklamia hominis*, molecular identification, *16S rRNA* sequencing, plant extract and essential oils susceptibility.

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INTRODUCTION

Facklamia spp. were named originally in honor of Dr. D.J. Facklam, who was a great microbiologist, and extensive taxonomical work by this microbiologist led to major advances in the definition and categorizing of *Streptococci*, notably including those that were not identified as the better-known Lancefield groups, H, I and K types. His work provided preliminarily information on the Genera such as *Streptococcus* and *Gemella*, which eventually gave way to the discovery of such a genus as *Facklamia* (Bastos *et al*.; 2024).

Gram-positive bacteria include a wide range of microorganisms, which are very important in human life, development of diseases, and the balance of nature. Major medically significant Genera found in this group are *Staphylococcus, Streptococcus* and *Enterococcus* and they all have a thick peptidoglycan layer which sustains the crystal violet during Gram staining (Jama *et al*.; 2025) Even though these Genera have become an object of vast research because of their influence on nosocomial and community infections, little has been done regarding atypical or emerging species in this category. Perhaps the most recent of them, *Facklamia* species are facultative anaerobic, catalase-negative, Gram-positive, coccoid organisms which produce alpha-hemolysis (Gahl *et al*.; 2020).

In the past, mis-identification of *Facklamia* species as phenotypically similar genera, like *Streptococcus* and *Enterococcus* has been an issue resulting in under-reporting and little clinical awareness. Nevertheless, the improvement of diagnostic techniques, such as *16SrRNA* gene sequencing and MALDI-TOF MS, has allowed the unambiguous identification of them, which led to the increased medical relevance of these agents (Church *et al*.; 2020).

Common species of *Facklamia* (including *F. hominis*, *F. ignava*, and *F. languida*) have been reported to be clinically isolated out of samples collected, and considered to include blood, cerebrospinal fluid, vaginal swabs, urine, and surgical site exudate (Rossi *et al*.; 2023).

Biochemically they resemble other non-Enterococcus Gram-positive cocci, posing a marked challenge to clinical diagnosis since leaving aside such conventional tests as catalase and bile-esculin; *Facklamia* species represent an indistinguishable entity. Nevertheless, the goal of exact identification is attributable to other distinct characteristics, including alpha-hemolysis, the absence of catalase, and molecular diagnostics (Cheung and Otto, 2023).

The new developments indicate that *Facklamia* spp. could have virulence factors such as the ability to form biofilm and evade immunity with implications of more antimicrobial resistance and their tendency to induce persistent infections that were hard to treat. These discoveries put forward an urgent necessity to estrange their genetics, the profiles of resistance, and their epidemiological impact, as they become more and more isolated in the clinical setting in many parts of the globe (Fotedar *et al*.; 2021; Gahl *et al*.; 2020).

The diagnostics and increased clinical response of *Facklamia*, especially *F. hominis*, highlights the need to conduct systematic studies. A detailed explanation of their virulence factors and patterns of susceptibility to plant-derived extracts may enhance accurate diagnosis, as well as better investment of effective therapeutic measures against the infections by these uncommon pathogens. (Mahmood and Essa, 2021).

MATERIALS AND METHODS

Collection of Samples and Pre-Analytical Processing:

The patients in this study were admitted in various hospitals located both east and west of Mosul/ Iraq on 20th December 2024 to 23rd March 2025, and their clinical fluid samples like urine, pus and blood. As for the swab samples, they were collected from the vaginal area using sterile swabs, swab from vaginal samples, were collected during the admission period. These were urine, blood, pus and high vaginal swab specimens of patients of suspected bacterial infections. Sterile sample collection was done using autoclaved bottles to reduce chances of contamination and better viability. They were immediately conveyed to the microbiology department at 4 o C and packed anaerobically to preserve the integrity of microbe isolates (Coelho et al.; 2022).

Microbiological culture and initial identification:

Upon arrival, samples were inoculated onto a range of culture media, including blood agar and MacConkey agar. Plates were incubated anaerobically at 37 °C for 18–24 hours, with slow-growing organisms given an additional 24 hours. Gram staining and biochemical screening, including catalase and oxidase assays, were performed for preliminary classification. Attention was drawn to 37 Gram-positive isolates showing atypical colony morphology (e.g.; α-hemolysis, minute colonies) or unexpected biochemical profiles. These isolates were initially associated with genera such as *Staphylococcus*, *Streptococcus*, *Enterococcus*, and *Lactobacillus*.

Presumptive identification of atypical isolates:

Among the 37 atypical Gram-positive bacteria, four isolates (2% of total samples) demonstrated phenotypic and biochemical features characteristic of the genus Facklamia. These isolates were catalase-negative, displayed alpha-hemolysis, and appeared as short chains of Gram-positive cocci.

Preliminary identification was performed based on colony morphology and standard biochemical profiling techniques (Arellano-Arellano et al.; 2024).

Selective media and cultivation conditions for *Facklamia*:

Isolation of *Facklamia* spp. requires selective, enriched media to support its fastidious growth, four blood-based agars were utilized: blood agar with 5% sheep or 10% horse blood, and Columbia agar with 10% horse or 5% sheep blood. These media were selected following prior studies demonstrating their suitability for cultivating various *Facklamia* species (Demir and Hazırolan, 2024). Plates were incubated aerobically at 37 °C for 24–48 hours. Facklamia colonies were distinguishable from other Streptococci by their weak or absent α-hemolysis.

Molecular confirmation of *Facklamia hominis*:

To perfectly identify the atypical isolates, partial 16SrRNA gene sequencing was undertaken as it is being administrated as the gold standard of bacteria species identification (Janda and Abbott, 2007). Universal primer PCR was done on genomic DNA Genomic DNA was extracted using a commercial kit (Geneaid, Taiwan) and a phylogenetic heatmap and evolutionary tree were constructed using MEGA v6.0 software:

- 27F (5′- AGAGTTTGATCMTGGCTCAG -3′)
- 1522R (5′- AAGGAGGTGATCCARCCGCA -3′).

Preparation of the Custom Culture Medium for *Facklamia hominis*:

Given the precise nutritional needs of F. hominis, a tailored culture medium was prepared. The formulation included:

- Beef extract peptone (nitrogen source)
- Tryptone (amino acids)
- Yeast extract (vitamins and growth factors)
- Sodium chloride (osmotic balance)
- Mineral salts (magnesium sulfate, calcium chloride)
- B-complex vitamins (commercial grade)

The medium was adjusted to pH 7.2 ± 0.1 using NaOH or HCl, then autoclaved at 121 °C for 15 minutes. After cooling to 40–45 °C, 5% packed RBCs from Mosul's blood bank were added. comparative media were prepared using equivalent percentages of horse or sheep blood. Plates were incubated under microaerophilic–anaerobic conditions with 5–10% CO_2 at 37 °C for 48–72 hours.

Colony characteristics, including density, size, morphology, color, margins, and α-hemolysis clarity, were systematically evaluated across the three media types. (Rossi et al.; 2023).

Preparation of plant extracts and essential oils and phytochemical screening

Plant materials were carefully sourced from trusted suppliers, rinsed in tap water, and then rinsed by sterile distilled water to eliminate surface contaminants. Samples were shade-dried at

ambient temperature for at least 7 days to preserve heat-sensitive phytochemicals (Ingle et al.; 2017) dried plant tissues were pulverized using sterilized electric grinders.

Ethanolic extracts were prepared by combining 20 g of dried plant powder with 200 mL of 70% ethanol in airtight flasks, stored at 4 °C for 24 hours with manual agitation 3–4 times daily (Wendakoon et al.; 2012). The mixtures were vacuum-filtered through sterile gauze, and solvents were evaporated at temperatures below 40 °C to avoid thermal degradation. Resulting dried extracts were sealed in sterile vials and stored at −20 °C until use. The concentration of the ethanolic extracts was 100 mg/cm³, while the concentration of the oils was 50 mg/cm³. Five plant-derived extracts were processed using identical protocols:

1. Pomegranate peels (Punica granatum)
2. Myrrh resin (Commiphora molmol)
3. Cinnamon bark (Cinnamomum verum)
4. Grape seeds (Vitis vinifera)
5. Propolis (bee resin)

Ethanolic Plant Extract Preparation Method (Paraphrased for Academic Use):

The selected plant materials (e.g.; pomegranate peels, myrrh, cinnamon bark, grape seeds, or Propolis) were thoroughly rinsed with tap water to remove surface impurities, followed by sterilized distilled water to ensure microbial decontamination. The cleaned samples were then air-dried in the shade or placed in a low-temperature oven (37–40˚C) to preserve thermolabile constituents. Once adequately dried, the materials were finely ground using a sterile electric or manual grinder to obtain a uniform powder. Exactly 20 grams of each powdered sample were weighed and transferred into a sterile glass flask containing 200 mL of 70% ethanol. The mixture was incubated in a refrigerator at 4˚C for 24 to 48 hours, with manual agitation performed three times per day to enhance the extraction of bioactive compounds. Following the maceration period, the extract was filtered through sterile medical gauze, then evaporated at temperatures not exceeding 40˚C to remove residual ethanol. The resulting dry extract was stored in sterile, light-resistant tubes at -20˚C until further use in antimicrobial bioassays. Each extract was prepared and stored under conditions optimized for bioactivity preservation and subsequent antimicrobial testing.

RESULTS AND DISCUSSION

Isolation and identification of *Facklamia hominis*

Out of 200 clinical fluid samples, four isolates (2%) were provisionally identified as *Facklamia hominis* based on colony appearance, Gram stain, and initial biochemical reactions. Positive and negative growth in addition to fungal growth, samples without growth were also found, as shown in (Figure 1). The isolates were distributed as follows:

Distribution of Clinical Isolates

Type of Microorganism	Percentage (%)
Gram-positive bacteria	18.5
Gram-negative bacteria	40
Facklamia hominis	2
Fungi (predominantly Candida spp.)	12.5
Negative samples	27

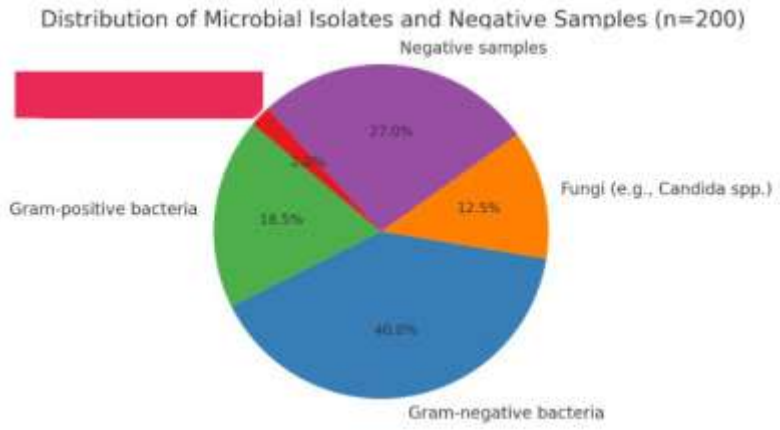

Fig. 1: Diagram shows the percentage of *Facklamia hominis* bacteria among the total isolated samples.

These strains were recovered from two urine and two high vaginal swab specimens. Molecular confirmation using partial 16SrRNA gene sequencing underscored the limitations of relying solely on conventional phenotypic methods when handling such fastidious or atypical microorganisms (Chakraborty *et al*.; 2021). This observation is in agreement with the existing reports that *F. hominis* could be perceived as a part of the vaginal microbiome with transient presence or appear as an opportunistic pathogen with specific clinical conditions. The outcome of the greatest increase in TGN was limited (Abat *et al*.; 2015; Schneidewind *et al*.; 2017). The findings pertain to the necessity of the implementation of molecular diagnostics, especially 16SrRNA analysis, into everyday clinical practice to detect the rarely observed or misidentified Gram-positive cocci incorrectly (Mahittikorn *et al*.; 2021).

Cultivation and selective media optimization:

Facklamia hominis was nutritionally fastidious and was slow growing requiring specialized and enriched culture media. Blood agar was inoculated with 5% sheep blood or 10% horse blood and Columbia agar were inoculated with the same percentage of blood. Despite the growth being slight, colonies produced poor or no α-hemolysis and thus differed significantly with the typical Streptococci. Remarkably, agar made of horse blood demonstrated better visibilities of hemolysis, which can be explained by its original biochemical composition, an observation that can be attributed to previous findings highlighting the role of blood source on the culture's performance (Schneidewind *et al*.; 2017).

In order to still further increase the growth, we prepared a special medium supplemented with a balanced mixture of amino acids, peptides, vitamins, minerals and ionic salts; also, the osmotic adjustments were made and the pH well controlled. This agar produced much larger, more apparent colonies with clear 2-hemolytic rings Fig. (2A) and this was an illustration of the importance of customizing culture media of fastidious clinical by the demonstration of it.

Justification for the custom-enriched medium:

In *Facklamia spp*.; nutritional and environmental needs are quite demanding and ordinary commercial media cannot always meet environmental and/or nutritional requirements. An enriched nutritive medium was specially developed by adding to standard nutrient broth an improved synthesis of amino acids, peptides, vital/ necessitate vitamins, mineral salts, and balanced electrolyte. These alterations would enhance pH and osmotic status, which promoted the strong erythrocyte-bacteria interactions. The resulting medium yielded colonies that were larger and clearer with clearer 8 hemolytic zones and made isolation much more accurate and diagnostic yield much higher. The people in the minority serve as the ones who dictate the definition and allow the majority of the population to define the minority and condition it (Nguyen *et al*.; 2022).

Impact of packed red blood cells (RBCs)

We made comparisons of three sources erythrocytes of horse blood, sheep blood and packed RBC. The conditions that gave the strongest results were packed RBC based media, which produced a higher clarity of hemolysis and bacterial loads. This can probably be attributed to three factors (1) increased concentration of erythrocytes, (2) lower plasma interference to the interaction of hemolysin, and (3) better batch-to-batch reproducibility Fig. (2B). These results coincide with those of the recent works propagating the blood component manipulation to fuel the specialized microbial growth (Livshits *et al*., 2021). The use of concentrated red blood cells (packed RBCs) with much of the plasma removed prepared via media had better results than the use of whole sheep or horse blood. This was seen to be improved by, increased hemolytic visibility, enhanced bacterial growth, and absorption of variability between growths. In our findings, it is evident to note that adaptation of culture media may be performed locally on the occasion of fastidious or unusual pathogens in clinical practice.

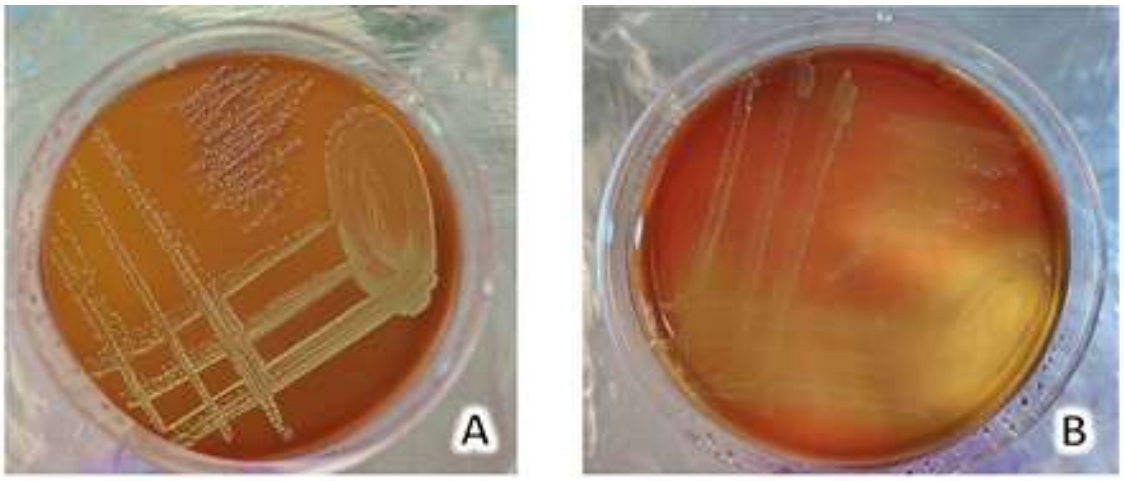

Fig. 2: (A) The dense growth demonstrates using locally manufactured media. (B) The (alpha) hemolysis obvious using packed RBCs.

Anaerobic cultivation using GasBag systems:

As observed in their earlier studies, here also, the successful use of sealed and tight, anaerobic containers such as Gas Pak or AnaeroPack allowed cultivation of the oxygen-sensi+tive *F. hominis* (Ruangrungrote et al.; 2008). Stable oxygen-limited environments were possible in these systems that were more cost effective and easy to operate as opposed to automated anaerobic chambers. The use of these in our study played a critical role in the isolation of this rare pathogen, and this clearly shows the relevance of using tailor-made cultivation framework in clinical microbiology (Jaques et al.; 2025).

Phylogenetic sequencing of 16s ribosomal RNA

Sequence analysis showed ≥99% identity to *Facklamia hominis* for all four strains. The obtained sequences were deposited in GenBank (Accession Nos. PV719694.1, PV719695.1, PV719696.1, and PV719697.1). This molecular approach not only confirmed species but also enabled phylogenetic comparisons with international counterparts, enhancing our understanding of evolutionary and clinical significance (Clarridge III, 2004).

The evaluation of genetic diversity and subsequent evolutionary relationship of *F. hominis* was able to provide a solid basis due to the partial sequencing of the 16SrRNA gene. The strains of eight countries globally were compared to our strains in Iraq, and the results of this comparison indicated a close genetic distance of 0.00-0.01, referring to a conserved phylogenetically lineage due to healthcare practices/ medical travel or global migration (Abuhilal, 2022). The results of visualization

were also demonstrated by heatmap and UPGMA clustering using Maximum Composite Likelihood and MEGA v6.0 to construct trees Fig. (3).

There were small substitutions in the nucleotides (T to C, at position 309 and G to A at 618); this occurred in our Iraqi strains which did not affect overall sequence identity, indicating regional genetic stability (Mostafa *et al*.; 2019).

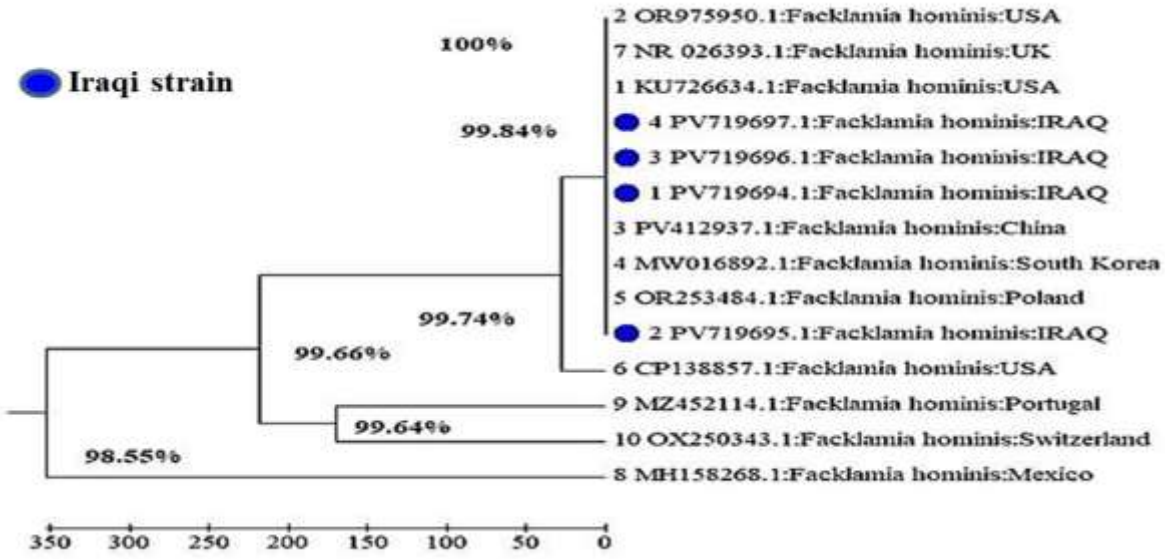

Fig. 3: Phylogenetic Tree Showing the Genetic Relationship of Iraqi *Facklamia hominis* Isolates with Global Strains Based on *16SrRNA* Sequences

Antibacterial activity of plant extracts:

Plant extracts demonstrated differential antibacterial efficacy against *F. hominis*, with ethanolic extracts consistently outperforming essential oils (Al-Sabaawy and Al-Layla.;2022). Ethanol effectively extracts bioactive compounds polyphenols, flavonoids, tannins known for antimicrobial activity (Daglia, 2012).

- Pomegranate peel ethanolic extract significantly inhibited *F. hominis* growth, likely due to its high content of ellagitannins (e.g.; punicalagin) and gallic acid, which disrupt bacterial cell walls and inactivate key metabolic enzymes (Al-Zoreky, 2009). In contrast, its essential oil showed minimal antibacterial activity, possibly due to the lower concentration of active phenolic. (Mousa and Jankeer, 2019)
- Propolis ethanolic extract demonstrated strong antibacterial activity, attributed to the synergistic action of flavonoids, phenolic acids, and CAPE, which destabilize bacterial membranes and inhibit protein synthesis and DNA replication (Al-Haider *et al*.; 2019). Its essential oil, however, exhibited limited effectiveness, potentially due to poor volatility or low diffusion through agar. (Al-Rassam *et al*.; 2008).
- Cinnamon ethanolic extract exhibited the highest activity of all the tested compounds mainly because Cinnamaldehyde and the eugenol are found to cause disintegration of the membrane and disrupts the bacterial enzyme system and nucleic acid synthesis (Singh *et al*.; 2007). The result of the essential oil was not as effective because it may be unstable or evaporates fast under normal incubation.
- Myrrh ethanolic extract had an inhibitory effect of a moderate to strong magnitude, which might be due to the presence of sesquiterpenes like furanoeudesma-1,3-diene and Curzerene, disrupting the integrity of the membranes of cells and the enzyme activity (Baek *et al*.; 2019). Its essential

oil showed fluctuating effects implying that there are specific active compounds that are more extractable in alcohol rather than oily preparations.

- Grape seed ethanolic extract had an intermediate activity, and it could be explained by the presence of polyphenols, such as proanthocyanidins and catechins, as they disrupt bacterial aerobic respiration and protein synthesis (Gupta *et al*.; 2020).

The essential oil form did not have remarkable antibacterial activities, as it might be attributed to hydrophilicity of active constituents, which were more soluble in polar solvent such as ethanol.

The antibacterial effect of ethanolic extracts showed significantly higher effective values when used with *Facklamia hominis* clinical isolates than their matching essential oils, with the extracts of pomegranate peel, Propolis and cinnamon being the most effective inhibitors. This increased bioactivity has been largely explained by the fact that ethanol has a greater ability to dissolve a broad range of polar phytochemicals including phenolic and flavonoids, indicatively underrepresented in the essential oil's fractions. As illustrated in (Table 2), The complex interplay between the plant origin, choice of extraction solvent, and resultant antibacterial efficacy highlights the critical importance of optimizing extraction methodologies to maximize therapeutic potential. Overall, the results underscore the potential of specific plant-derived ethanolic extracts, particularly those from pomegranate, Propolis, and cinnamon, as effective natural antibacterial agents against *F. hominis*.

Furthermore, they highlight the critical role of extraction method in preserving and delivering bioactive compounds.

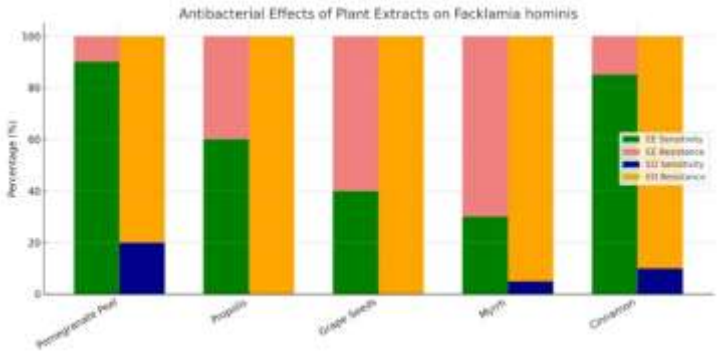

Fig. 4: Comparative Antibacterial Activity of Ethanolic Extracts and Essential Oils from Medicinal Plants Against *Facklamia*

CONCLUSIONS

This study successfully isolated *Facklamia hominis* from clinical samples using optimized culture conditions and confirmed its identity through 16SrRNA gene sequencing. The isolates showed high genetic stability and low global variation, indicating strong ecological adaptation. Ethanolic extracts from pomegranate peel, cinnamon, and Propolis demonstrated strong antibacterial activity against *F. hominis*, suggesting their potential as alternative natural treatments. The findings emphasize the need for improved molecular diagnostics and tailored growth media to accurately identify and treat rare and fastidious pathogens like *F. hominis*.

REFERENCE

Abat, C.; Garcia, V.; and Rolain, J. (2015). *Facklamia hominis* scapula abscess, Marseille, France. New Microbes and New Infections, **9**(13). DOI: 10.1016/j.nmni.2015.11.003

Abuhilal, A. E. K. (2022). Comparison between cultivation and 16sRNA sequencing based-approaches analysis for bacteria in urine and cerebrospinal fluid Al-Quds University].

Al-Haider, S. M.; Al-Niaeem, K. S.; Resen, A. K. (2019). Prevalence and antibiotic resistance in Aeromonas species isolated from common carp (Cyprinus carpio L.) cultivated in floating cages at Al-Hilla river. *Basrah J. Agr. Sci*., **32**(2), 33-43. DOI:10.37077/25200860.2019.194

Al-Rassam, Z. T.; Al-Tobje, M. A.; Shareef, A. Y. (2008). The Study of Inhibitory Effect of Propolis on Some Gram positive and Gram-Negative Bacteria. *Raf. J. Sci*., **19**(2). DOI: 10.33899/rjs.2008.41569

AL-Sabaawy, A. M.; AL-Layla, E. A. M. (2024). Antibacterial activity of the aqueous and oily extracts of some plants against strains of *Staphylococcus* spp. and *Pseudomonas aeruginosa* from chalazion. *Malaysian J. Mic. bio*. **20**(2), 119-124. doi.org/10.21161/mjm.240018

Al-Zoreky, N. S. (2009). Antimicrobial activity of pomegranate (*Punica granatum* L.) fruit peels. *Inter. J. food. Mic. bio*., **134**(3), 244-248. DOI:10.1016/j.ijfoodmicro.2009.07.002

Arellano-Arellano, C.; Villeda-Gabriel, G.; Prado-Galbarro, F.-J.; Barrientos González, P. A.; Reyes Apodaca, M.; Gomez-Ramirez, U.; Villanueva García, D.; Contreras-Rodríguez, A.; Aguilera-Arreola, M. G.; Franco Hernández, M. I. (2024). Prevalence of atypical bacteria in patients from different pediatric age groups diagnosed with a respiratory disease. *Mic.Org.*, **12**(11), 2328. DOI: 10.3390/microorganisms12112328

Barajas, H. R.; Romero, M. F.; Martínez-Sánchez, S.; Alcaraz, L. D. (2019). Global genomic similarity and core genome sequence diversity of the Streptococcus genus as a toolkit to identify closely related bacterial species in complex environments. *Peer. J.*, **6**, e6233. DOI: 10.7717/peerj.6233

Baek, S. J.; Kim, D. H. (2016). The Study on anti-obesity of Myrrh ethanol extract. *Korea J. Her*., **31**(4):11-18. DOI:10.6116/kjh.2016.31.4.11

Bastos, M. M. F.; Maran, B. M.; Maran, E. M.; Miotto-Lindner, M.; Verruck, S. (2024). Assessment of microbial ecology in artisanal salami during maturation viametataxonomic analysis. *Food Sci. Tech.,* **44**. DOI: 10.5327/fst.00147

Chakravorty, S.; Helb, D.; Burday, M.; Connell, N.; Alland, D. (2007). A detailed analysis of 16S ribosomal RNA gene segments for the diagnosis of pathogenic bacteria. *J. mic. bio*. *Meth*., **69**(2), 330-339. DOI:10.1016/j.mimet.2007.02.005

Cheung, G. Y.; Otto, M. (2023):Virulence mechanisms of staphylococcal animal pathogens. *Inter. J. Mol. Sci*., **24**(19), 14587. DOI:10.3390/ijms241914587

Church, D. L.; Cerutti, L.; Gürtler, A.; DOI:10.3390/ijms241914587Griener, T.; Zelazny, A.; Emler, S. (2020). Performance and application of 16S rRNA gene cycle sequencing for routine identification of bacteria in the clinical microbiology laboratory. *Clin. Mic. Bio. reviews*, **33**(4), 10.1128/cmr. 00053-00019. DOI:10.1128/CMR.00053-19

Clarridge III, J. E. (2004). Impact of 16SrRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clin. Mic. Bio. Reviews*, **17**(4), 840-862. DOI:10.1128/CMR.17.4.840-862.2004

Coelho, M. C.; Malca, F. X.; Silva, C. C. (2022). Lactic acid bacteria in raw-milk cheeses: From starter cultures to probiotic functions. *Foods*, **11**(15), 2276. DOI:10.3390/foods11152276

Demir, M.; Hazırolan, G. (2024). Rapid bacterial identification from positive blood cultures by MALDI-TOF MS following short-term incubation on solid media. *Inf. Dise. Clin. Mic. Bio.*, **6**(2), 141. DOI:10.36519/idcm.2024.31

Daglia, M. (2012). Polyphenols as antimicrobial agents. *Curr. Opini. bio. Tech*., **23**(2), 174-181. DOI:10.1016/j.copbio.2011.08.007

Fotedar, R.; Sankaranarayanan, K.; Caldwell, M. E.; Zeyara, A.; Al Malki, A.; Kaul, R.; Al Shamari, H.; Ali, M.; Al Marri, M.; Lawson, P. A. (2021). Reclassification of *Facklamia ignava*, *Facklamia sourekii* and *Facklamia tabacinasalis* as *Falseniella ignava* gen. nov.; comb. nov.; *Hutsoniella sourekii* gen. nov.; comb. nov.; *Ruoffia tabacinasalis* gen. nov.; comb. nov.; and description of *Ruoffia halotolerans* sp. nov.; isolated from hypersaline Inland Sea of Qatar. *Ant. van Leeuwenhoek*, **114**, 1181-1193. DOI:10.1007/s10482-021-01587-7

Gahl, M.; Stöckli, T.; Fahrner, R. (2020). *Facklamia hominis* bacteremia after transurethral resection of the prostate: a case report. *BMC Urology*, **20**, 1-4. DOI:10.1186/s12894-020-00762-8

Gupta, M.; Dey, S.; Marbaniang, D.; Pal, P.; Ray, S.; Mazumder, B. (2020). Grape seed extract: Having a potential health benefits. *J. Food Sci. Tech*., **57**,1205-1215. DOI:10.1007/s13197-019-04113-w

Ingle, K. P.; Deshmukh, A. G.; Padole, D. A.; Dudhare, M. S.; Moharil, M. P.; Khelurkar, V. C. (2017). Phytochemicals: Extraction methods, identification and detection of bioactive compounds from plant extracts. *J. Pharma. Phyt. chem*., **6**(1)32-36.

Jama-Kmiecik, A.; Mączyńska, B.; Frej-Mądrzak, M.; Choroszy-Król, I.; Dudek-Wicher, R.; Piątek, D.; Kujawa, K.; Sarowska, J. (2025). The changes in the antibiotic resistance of *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Enterococcus faecalis* and *Enterococcus faecium* in the clinical isolates of a multiprofile hospital over 6 Years (2017–2022). *J. Clin. Med*., **14**(2), 332. DOI:10.3390/jcm14020332

Janda, J. M.; Abbott, S. L. (2007). 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: Pluses, perils, and pitfalls. *J. Clin. Mic. Bio*., **45**(9), 2761-2764. DOI:10.1128/JCM.01228-07

Jaques, A.; Ireland, M.; Van der Land, C.; Dirmeier, R.; Williamson, N.; Christgen, B. (2025). Experimental procedures for studying microbial reactions under high hydrogen gas saturations in microcosms. *Methods X*, **14**, 103344. DOI:10.1128/JCM.01228-07

Livshits, L.; Barshtein, G.; Arbell, D.; Gural, A.; Levin, C.; Guizouarn, H. (2021). Do we store packed red blood cells under "Quasi-Diabetic" conditions? *Biomol.*, **11**(7), 992. DOI:10.3390/biom11070992

Mahmood, M.; Essa, M. (2021). Antimicrobial activity of peptides extracted from camels' blood neutrophils against some pathogenic bacteria. *Iraqi J. Veter. Sci*., **35**(1), 33-37. DOI: 10.33899/ijvs.2020.126239.1270

Mahittikorn, A.; Udonsom, R.; Koompapong, K.; Chiabchalard, R.; Sutthikornchai, C.; Sreepian, P. M.; Mori, H.; Popruk, S. (2021). Molecular identification of *Pentatrichomonas hominis* in animals in central and western Thailand. *BMC vet. Res*., **17**(1), 203. DOI:10.1186/s12917-021-02904-y

Mousa, R. M.; Jankeer, M. H. (2019). The protective effect of pomegranate and lemon juices in number of biochemical parameters in blood serum and tissue of local rabbits infected by experimental urolithiasis. *Raf. J. Sci*., **28**(4). DOI: 10.33899/rjs.2019.163299

Mostafa, H. H.; Taffner, S. M.; Wang, J.; Malek, A.; Hardy, D. J.; Pecora, N. D. (2019). Genome sequence of a Facklamia hominis isolate from a patient with urosepsis. *Mic. Res. Anno*., **8**(17), 10.1128. DOI:10.1128/MRA.00100-19

Nguyen, M. H.; Clancy, C. J.; Pasculle, A. W.; Pappas, P. G.; Alangaden, G.; Pankey, G. A.; Mylonakis, E. (2019). Performance of the T2Bacteria panel for diagnosing bloodstream infections: a diagnostic accuracy study. *Annals Inte. Med*., **170**(12), 845-852. DOI:10.7326/M18-2772

Rossi, F.; Santonicola, S.; Amadoro, C.; Marino, L.; Colavita, G. (2023). Recent records on bacterial opportunistic infections via the dietary route. *Microorg.*, **12**(1), 69. DOI:10.3390/microorganisms12010069

Ruangrungrote, S.; Intasorn, A.; Sindermsuk, J.; Attasongkroah, M. (2008). The feasibilitv of GasPak envelope production for anaerobic bacteria cultivation. *Sci. Tec. Asia*, 1-7, DOI: org/index.php/SciTechAsia/article/view/41427

Schneidewind, L.; Kranz, J.; Schlager, D.; Barski, D.; Mühlsteadt, S.; Grabbert, M.; Queissert, F.; Frank, T.; Pelzer, A. E. (2017). Mulitcenter study on antibiotic prophylaxis, infectious complications and risk assessment in TUR-P. *Cen. Eur. J. Uro*., **70**(1), 112. DOI:10.5173/ceju.2017.941

Singh, R. J.; Chung, G. H.; Nelson, R. L. (2007). Landmark research in legumes. *Genome*, **50**(6), 525-537. DOI:10.1139/G07-037

Taher, E. M.; Abdullah, B. A.; Taie, A. A. A. (2024). Phenotypic and genotypic detection of biofilm genes for *Proteus*, *Morganella*, *Providencia* (PMP) bacterial group isolated from clinical samples of some hospitals in Mosul/Iraq. *J. App. Natu. Sci*., **16**(4), 1719. DOI: 10.31018/jans.v16i4.5935

Tamura, K.; Nei, M.; Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Pro. Nat. Acad. Sci*., **101**(30), 11030-11035. DOI:10.1073/pnas.0404206101

Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A.; Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol. Bio Evo*., **30**(12), 2725-2729. DOI:10.1093/molbev/mst197

Wendakoon, C.; Calderon, P.; Gagnon, D. (2012). Evaluation of selected medicinal plants extracted in different ethanol concentrations for antibacterial activity against human pathogens. *J. Med. Act. Plants*, **1**(2), DOI:10.7275/R5GH9FV2

حساسية المستخلصات النباتية الإيثانولية والتوصيف الجزيئي لبكتيريا *Facklamia hominis* مُمرض ناشئ وغير نمطي موجب لصبغة غرام

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الملخص

هدفت هذه الدراسة إلى الكشف عن وجود بكتيريا موجبة لصبغة كرام غير النمطية وتقييم حساسيتها تجاه مستخلصات نباتية مختارة، من خلال تحليل 200 عينة سريرية متنوعة جُمعت من مرضى في عدد من مستشفيات مدينة الموصل/العراق. شملت العينات كلاً من الدم، القيح، المسحات المهبلية، والبول. جرى عزل وتشخيص البكتيريا باستخدام تقنيات ميكروبيولوجية معيارية. أظهرت النتائج تنوعاً كبيراً في أنواع البكتيريا المعزولة، وشملت بكتيريا موجبة وسالبة لصبغة غرام، بتوزيعات مختلفة حسب مصدر العينة. من بين العزلات، كانت 37 عزلة موجبة لغرام، وتضمنت أنواعاً شائعة من أجناس *Staphylococcus*، و*Streptococcus*، و*Enterococcus*، و*Lactobacillus*. أما العزلات السالبة لغرام، فتضمنت *Escherichia coli*، و*Proteus* spp، و*Klebsiella* spp، و *Pseudomonas* spp، إلى جانب عزلات فطرية محدودة تمثلت بشكل رئيسي بجنس Candida. وأظهرت بعض العينات عدم وجود نمو ميكروبي.

من بين هذه العزلات، تم تحديد أربع عزلات نادرة تعود لجنس *Facklamia*، وبالتحديد نوع *Facklamia hominis*، حيث ظهرت أربع مرات فقط في عينتين بول ومسحتين مهبليتين، وتم تأكيدها باستخدام التحليل الجزيئي، من خلال التقييم الظاهري، وتفاعل PCR نوعي، وتسلسل جين SrRNA16، مما يمثل أول توثيق لعزلات سريرية من *F. hominis* في العراق. وقد تم إيداع هذه التسلسلات في قاعدة بيانات GenBank (أرقام الدخول PV719694.1, PV719697.1, PV719696.1,PV719695.1 كشفت النتائج الجينومية المقارنة عن تشابه كبير مع العزلات العالمية، مع وجود طفرات محلية قد تعكس عمليات تكيّف بيئي.

أظهر اختبار الحساسية لمستخلصات النباتات تبايناً في درجات المقاومة، مما يشير إلى وجود تنوع جيني ومرونة تطورية لدى *F. hominis*. وتُعد هذه النتائج ذات أهمية سريرية في ضوء تصاعد ظهور هذا النوع البكتيري، مما يبرز الحاجة إلى تقنيات تشخيص جزيئية للكشف عن العوامل الممرضة النادرة والناشئة.

الكلمات الدالة: *Facklamia hominis*، التشخيص الجزيئي، تسلسل جين 16SrRNA، حساسية المستخلصات النباتية.