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Research Article

Molecular Detection and Phylogenetic Analysis of *Klebsiella aerogenes* in Diabetic Foot Ulcers in Iraq

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About Article

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ABSTRACT

Klebsiella aerogenes is a nosocomial bacterium which recognized increasingly as a significant contributor to the complexity and recalcitrance of diabetic foot ulcers. The study looks at molecular phylogenetic profiling of *K. aerogenes* in diabetic foot ulcers and estimating the relationship of infection to some related risk factors. Swab samples were collected from foot ulcer of an overall 143 diabetic patients at Al-Qadisiyah province, and subjected to molecular examination using the conventional PCR assay. Then, some positive *K. aerogenes* isolates were sent for sequencing, and the received data were submitted to the NCBI-GenBank database, and then analysed phylogenetically using the MEGA-11 software. Information related to age and sex of study patients as well as type of diabetes and glycemic control had been recorded as risk factors. Totally, there were 13.29% positive foot ulcers to *K. aerogenes* infection. Sequencing data and pahylogenetic analysis of 10 study *K. aerogenes* isolates revealed the presence of similarity (*) at range of 99.25-99.86% and substitution / mutation at range of 0.0001-0.0014% with the global GenBank-BLAST *K. aerogenes* isolates / strains; in particular Polish *K. aerogenes* strain (ID: OM250432.1). For risk factors, positive *K. aerogenes* infection was observed significantly in patients aged 30-50 (11.86%) and >50 (14.63%) years more than <30 (0%) years; males (14.66%) more than females (7.41%); type 2 diabetes (14.17%) more than type 1 (6.25%); and glycemic uncontrolled (17.07%) more than glycemic controlled (8.2%) patients. Subsequently, the risk of *K. aerogenes* infection was elevated significantly in patients aged >50 years more than 30-50 years and <30 years; males more than females; type 2 diabetes more than type 1; and glycemic uncontrolled more than controlled patients. This might represent the first Iraqi molecular phylogenetic study implicates *K. aerogenes* in diabetic foot ulcers indicating the need for moreover studies. Also, the nuanced interplay between bacterial virulence factors, host immune responses and the development of antimicrobial resistance necessitates a re-evaluation of current diagnostic and therapeutic paradigms for diabetic foot ulcers.

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1. INTRODUCTION

The genus of Klebsiella encompasses a diverse group of Gram-Negative bacterial species that widely distributed in natural environments and as a commensal within the human microbiota (Dong *et al.*, 2022; Madhi *et al.*, 2024). Among these species, *K. aerogenes*, previously known Enterobacter aerogenes, has garnered significant attention as a major opportunistic pathogen, which increasingly recognized for its role in nosocomial infections and propensity to multi-drug resistance (Wesevich *et al.*, 2020). Also, the bacterium characterized by presence of large polysaccharide capsule as a key virulence factor contributing to its pathogenicity and enables the bacterium to evade phagocytosis and contributes to biofilm formation that enhancing its survival and persistence within various hosts (Ibraheim *et al.*, 2024; Liu *et al.*, 2025). The intrinsic resistance of *K. aerogenes* to certain antimicrobial agents combined with its capacity to form biofilms significantly complicates the treatment strategies particularly in infections associated with indwelling medical devices (Russo *et al.*, 2023; Almatroudi, 2025).

2. LITERATURE REVIEW

Diabetes mellitus is a harmful metabolic and chronic illness characterized by persistently high blood glucose levels due to inadequate insulin production or when the body fails to utilize the produced insulin (Al-Shaeli *et al.*, 2022a, b). This persistent hyperglycemia is associated with long-term damage, dysfunction, and eventual failure of various organs including the eyes, kidneys, nerves, heart, and blood vessels (Mezil & Abed, 2021). Subsequently, the pervasive condition significantly impacts global health leading to serious macrovascular and microvascular complications that diminish quality of life and increase mortality rate (Zakir *et al.*, 2023). Globally, diabetes mellitus affects a substantial portion of the population, with type 2 alone impacting approximately 462 million individuals and contributing to over one million deaths in 2017, underscoring its significant public health burden (Erhunmwunse *et al.*, 2025). Diabetic foot ulcer represents a multifactorial complication in patients with diabetes mellitus, which is often leading to severe morbidity and mortality due to impaired wound healing and rapidly progressing to deeper tissues, necessitating surgical intervention or amputation if not promptly and appropriately managed (Chen *et al.*, 2023; Yachmaneni *et al.*, 2023). Given the poly-microbial nature often characterizing these infections, identifying the role of each specific pathogen is crucial for guiding effective therapeutic strategies (Chang & Nguyen, 2021; Jaber *et al.*, 2025).

In Iraq, although Gram-positive bacteria such as Staphylococcus aureus (Hussein and Saleh, 2024; Aifari, 2025), Streptococcus spp. (Habeeb *et al.*, 2021; Matar & Saleh, 2024), and Enterococcus spp. (Habeeb *et al.*, 2021; Turkei & Al-Dulaimi, 2024) in diabetic foot ulcers were analyzed extensively, few available data were identified which focused on traditional isolation of Gram-negative bacteria like Pseudomonas aeruginosa (Alkhudhairy *et al.*, 2020; Abd Khader & Raad, 2025) and K. pneumonia (Habeeb *et al.*, 2021; Matar & Saleh, 2024). Hence, this might represent the first study in Iraq aims to molecular phylogenetic profiling of *K. aerogenes* in diabetic foot ulcers with documenting of study isolates in the NCBI-GenBank database and estimating the relationship of infection to some related risk factors including age, sex, type of diabetes, and glycemic control.

3. METHODOLOGY

3.1. Ethical approval

The Scientific Committee in the Department of Pathological Analyses (College of Science, University of Al-Qadisiyah) was licensed this work.

3.2. Samples

Totally, 143 diabetic patients with foot ulcer of different ages and sexes who attended to the private medical clinics in Al-Qadisiyah province were selected to the present study, and subjected to obtaining of swab samples. Then, the collected swabs were transported using labeled plastic tubes containing 1ml of tryptone (15%) to be kept frozen (-20°C) for later testing by molecular tool. Data related to study patients including age, sex, type of diabetes, and glycemic control were recorded to estimated their relationships to positive infections.

3.3. Molecular testing

After preparation of swab samples, DNAs were extracted following the manufacturer instructions of the PrestoTM Mini gDNA Bacteria Kit (Geneaid, Taiwan) and examined then by the Nanodrop system (Thermo-Scientific, UK) to evaluate the concentration and purity of extracted DNAs. Targeting 16S rRNA gene, one set of primers was designed based on the NCBI-GenBank isolate (ID: NR_102493.2) and provided (F: 5′-TAC TCG CAG AAG AAG CAC CG-′3 and R: 5′-AGT TGC AGA CTC CAA TCC GG-′3) to be served for preparing the MasterMix tubes following the manufacturer instructions of the Accupower® PCR PreMix (Bioneer, Korea) at a final volume of 25µL for DNAs amplification in the Thermal Cycler system (BioRad, USA). Electrophoresis of agarose-gel (1.5%) stained with ethidium bromide was done at 100V and 80Am for 90min, and the positive bands were detected at an approximately 836bp using the UV transilluminator system (Clinx Science, China).

The DNAs of some positive isolates were sequenced, and submitted in NCBI-GenBank database. For phylogeny, MEGA-11 software and NCBI-Multiple Sequence Alignment (MSA) Viewer were served to analyzing the study *K. aerogenes* isolates by the ClustralW Alignment, Homology Sequence Identity and Phylogenetic Tree Analysis to indicate identity between the study *K. aerogenes* isolates and NCB-BLAST *K. aerogenes* isolates / strains.

3.4. Statistical analysis

GraphPad Prism Software was applied using the t-test, One-Way ANOVA in addition to Odds-Ratio (OR) and Relative Risk (RR) to detect significant differences between the obtained results at $p<0.05$. The confidence interval (95%CI) was calculated between the obtained data of various age groups (Al-Ethafa *et al.*, 2025; Al-Shaeli *et al.*, 2025).

4. RESULTS AND DISCUSSION

Among totally 143 diabetic patients, 19 (13.29%) swabs of foot ulcers were positively infected by *K. aerogenes* using the conventional PCR assay (Figure 1).

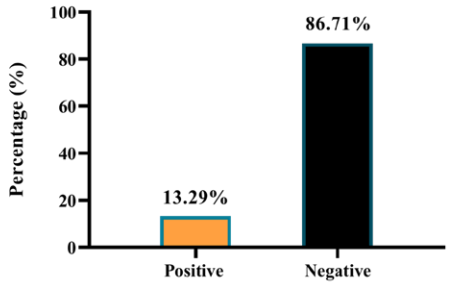

Figure 1. Total results for testing 143 swab ulcers by PCR

The sequencing data of 10 study *K. aerogenes* isolates were recorded in the NCBI-GenBank under the names of *Klebsiella aerogenes* isolate RAH1 to RHA10. Then, phylogenetic analysis of study *K. aerogenes* isolates revealed the presence of similarity (*) at range of 99.25-99.86% and substitution / mutation at range of 0.0001-0.0014% with the global GenBank-BLAST *K. aerogenes* isolates / strains. Comparative homology identity, phylogenetic tree analysis and MSA had shown an identity between the study *K. aerogenes* isolates and Polish *K. aerogenes* strain (ID: OM250432.1), (Figures 2-4, Table 1).

Figure 2. MSA for the study and global NCBI-GenBank *K. aerogenes* isolates / strains using the MEGA-11 Software

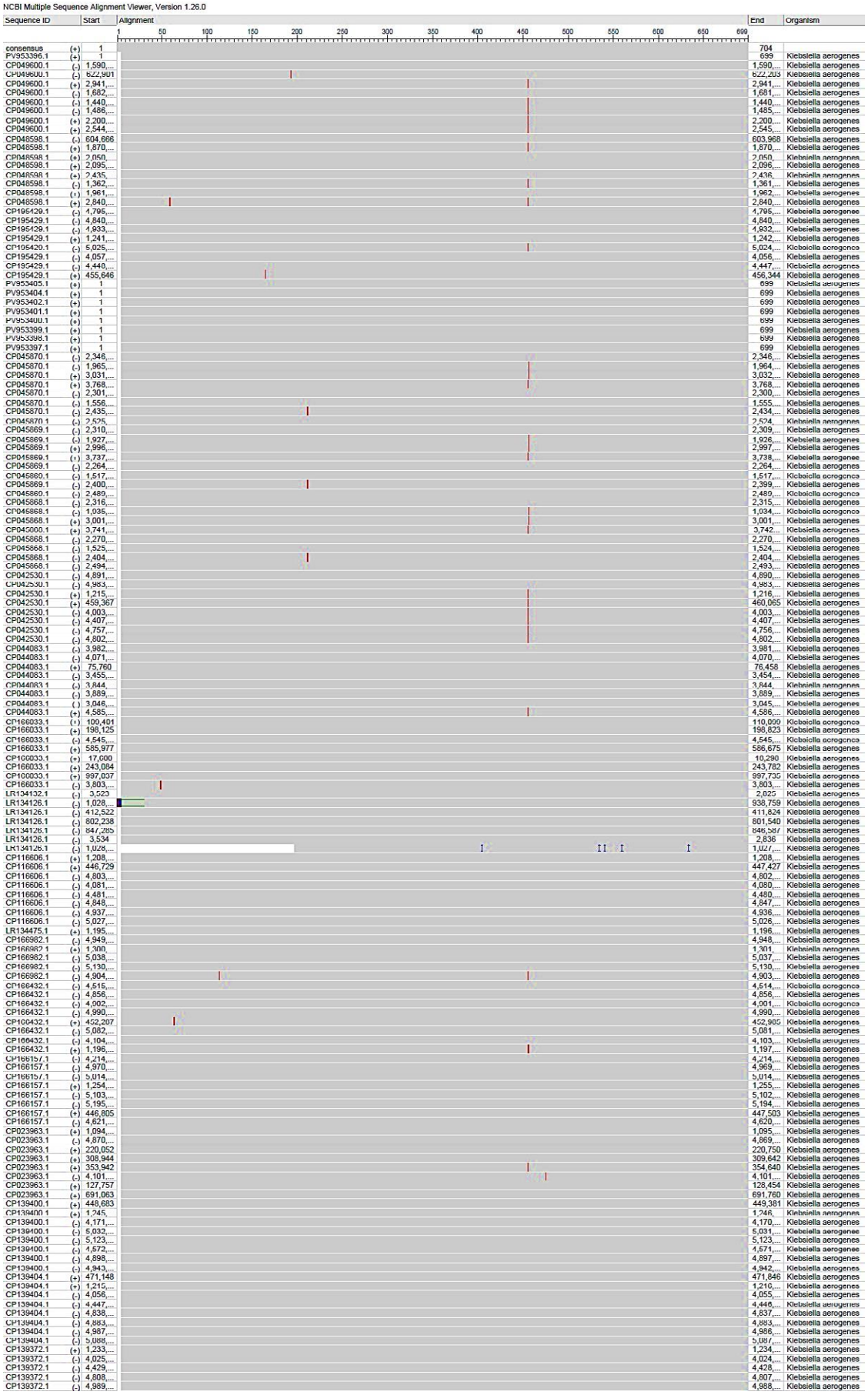

Figure 3. MSA for the study and global NCBI-GenBank *K. aerogenes* isolates / strains using the NCBI-MSA Viewer

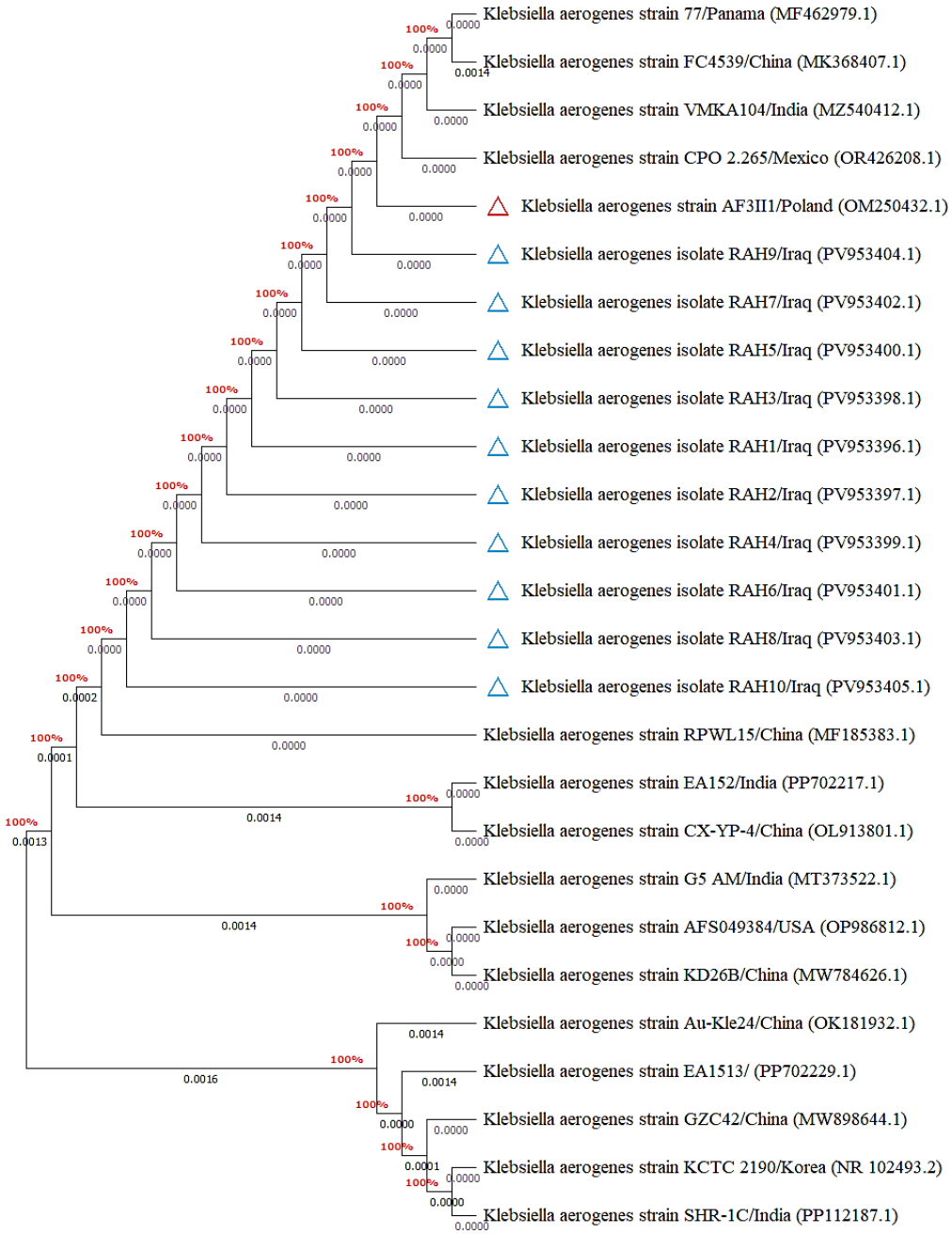

Figure 4. Phylogenetic tree analysis of study local and NCBI-BLAST *K. aerogenes* isolates / strains

Table 1. Homology sequence identity for study and global *K. aerogenes* isolate / strain

Local isolate		**NCBI-BLAST strain**				Identity (%)
Name	**Access. No.**	**Strain**	**Source**	**Country**	**Access. No.**	
RAH1	PV953396.1	AF3II1	Soil	Poland	OM250432.1	99.86
RAH2	PV953397.1	AF3II1	Soil	Poland	OM250432.1	99.73
RAH3	PV953398.1	AF3II1	Soil	Poland	OM250432.1	99.55
RAH4	PV953399.1	AF3II1	Soil	Poland	OM250432.1	99.79
RAH5	PV953400.1	AF3II1	Soil	Poland	OM250432.1	99.84
RAH6	PV953401.1	AF3II1	Soil	Poland	OM250432.1	99.25
RAH7	PV953402.1	AF3II1	Soil	Poland	OM250432.1	99.46

RAH8	PV953403.1	AF3II1	Soil	Poland	OM250432.1	99.51
RAH9	PV953404.1	AF3II1	Soil	Poland	OM250432.1	99.80
RAH10	PV953405.1	AF3II1	Soil	Poland	OM250432.1	99.43

For risk factors, significant elevation (p<0.0313; 95%CI: 10.48 to 28.14) in percentage of *K. aerogenes* infection among the study groups of age factor was observed in 30-50 years [11.86% (7/59)] and >50 years [14.63% (12/82)] when compared to <30 years [0% (0/2), (Figure 5).

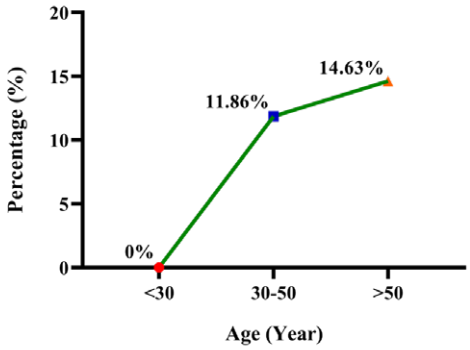

Figure 5. Total results of positive *K. aerogenes* among study groups of age factor

However, the risk of *K. aerogenes* infection (OR and RR) was elevated significantly (p<0.0001; 95%CI: 0.9399 to 23.55; and 95%CI: 0.9019 to 23.03, respectively) patients aged >50 years (1.315 and 1.27, respectively) when compared to 30-50 (0.808 and 0.832, respectively) as well as <30 years (0 and 0, respectively), (Figure 6).

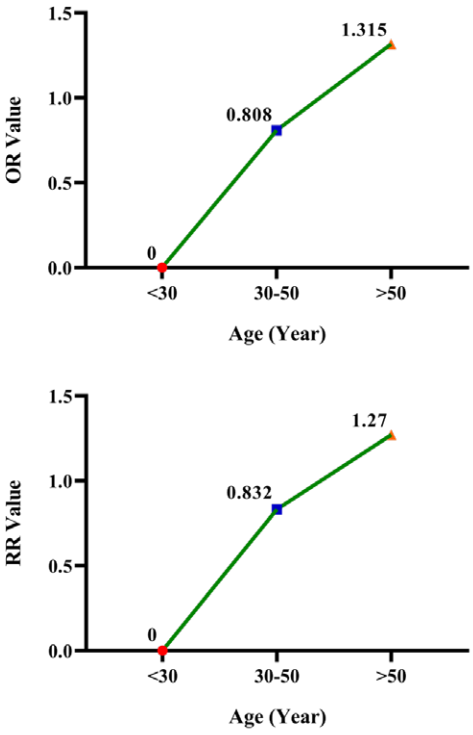

Figure 6. Results of infection risk (OR; RR) among the study groups of age factor

Significantly (p<0.0402; 95%CI: 35.02 to 57.09), positive *K. aerogenes* infections were higher in males [14.66% (17/116)] than females [7.41% (2/27)], (Figure 7). In addition, the risk of infection (OR; RR) was elevated significantly (p<0.0001; 95%CI: 0.9397 to 12.01; and 95%CI: 0.8183 to 10.67, respectively) in males (2.15 and 1.987, respectively) than females (0.465 and 0.503, respectively), (Figure 8).

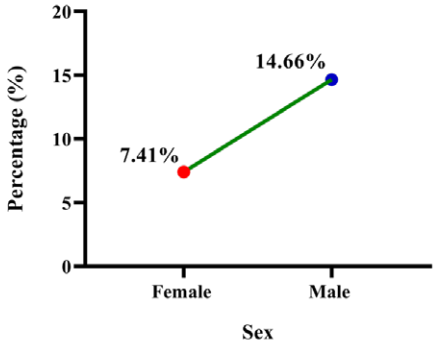

Figure 7. Total results of positive *K. aerogenes* among study groups of sex factor

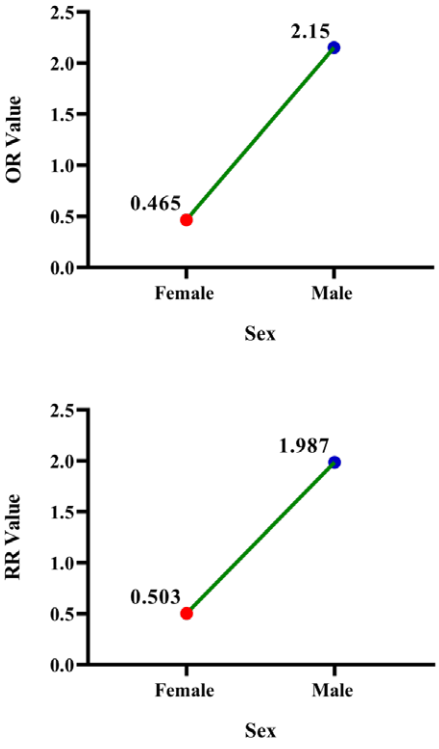

Figure 8. Results of infection risk (OR; RR) among the study groups of sex factor

According to type of diabetes mellitus, the findings were seen a significant association (p<0.0355; 95%CI: 40.11 to 60.53) of positive *K. aerogenes* infections to type 2 diabetes [14.17% (18/127)] more than type 1 [6.25% (1/16)] (Figure 9). Also, the

risk *K. aerogenes* (OR; RR) was noticed significantly (p<0.0001; 95%CI: 0.9163 to 14.50; and 95%CI: 0.9280 to 12.99, respectively) in type 2 (2.463 and 2.272, respectively) more than type 1 (0.406 and 0.44, respectively), (Figure 10).

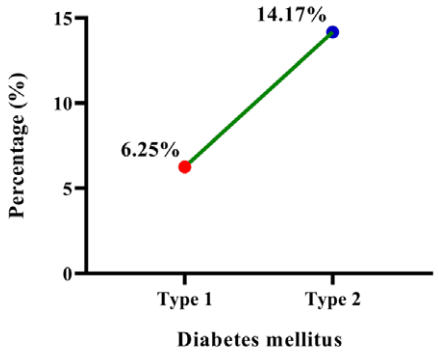

Figure 9. Total results of positive *K. aerogenes* according to type of diabetes mellitus

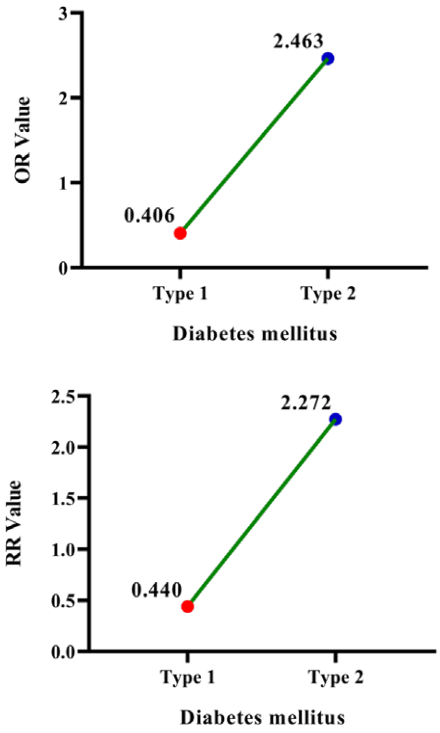

Figure 10. Results of infection risk (OR; RR) according to type of diabetes mellitus

Relation to glycemic control, positive *K. aerogenes* infections were increased significantly (p<0.0296; 95%CI: 43.72 to 68.99) in diabetes mellitus patients of uncontrolled [17.07% (14/82)] more than those of controlled [8.2% (5/61)] diabetes mellitus (Figure 11). Also, the risk of *K. aerogenes* infection was significantly higher (p<0.0001; 95%CI: 0.9509 to 13.34; and 95%CI: 0.8914 to 11.48, respectively) in glycemic uncontrolled patients (2.315 and 2.085, respectively) more than glycemic controlled individuals (0.432 and 0.48, respectively) (Figure 12).

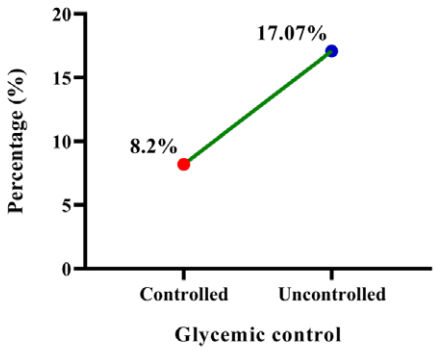

Figure 11. Total results of positive *K. aerogenes* according to glycemic control

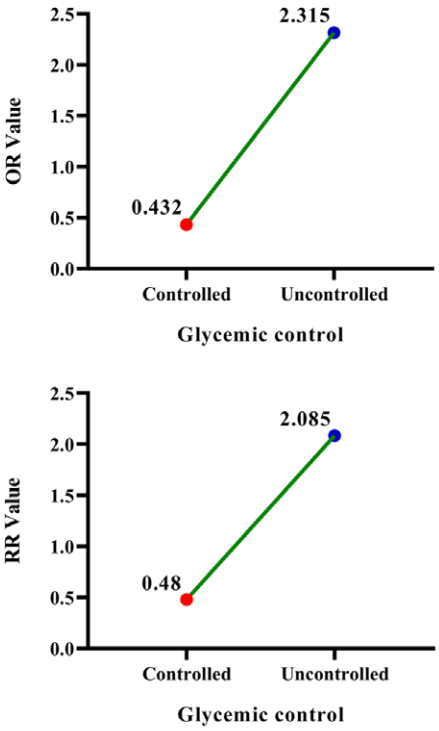

Figure 12. Results of infection risk (OR; RR) according to glycemic control

4.1. Discussion

Among the species of Klebsiella genus, though *K. aerogenes* is historically considered less virulent than K. pneumoniae, it is increasingly recognized for its involvement in opportunistic infections, particularly in immunocompromised individuals and those with chronic conditions such as diabetes, where it can acquire resistance to multiple antibiotics (Rajagopalan, 2005; Rossi *et al.*, 2023; Morgado *et al.*, 2024). In this study, 13.29% of diabetic foot ulcers were positive to *K. aerogenes* molecularly by the PCR assay as identified in Nigeria by Orji *et al.* (2009) who found that the prevalence rate of *K. aerogenes* in diabetic foot ulcers was 12.3%. In a number of studies, distribution of *K. aerogenes* had been investigated previously in the West of

Scotland neurosurgical unit (Price & Sleigh, 1970), as well as in the ward environments of a hospital and hands of nursing staff (Cooke *et al.*, 1979). In last study, authors have been suggested that there is large reservoir of *K. aerogenes* both in the bowel of patients and the environment. In recent studies, *K. aerogenes* was detected in 31.2% of 285 patients with bacteraemia (Álvarez-Marín *et al.*, 2021) as well as in 6.9% of hospitalized patients with blood stream infections (Guedes *et al.*, 2024). Phylogenetic analysis of study isolates demonstrated its identity with Polish *K. aerogenes* strain that isolated from the soil. Although, many studies have mentioned to the capability of this organism for persisting in various environments including soil, no specify exact causes for this ability (Glushakova *et al.*, 2022; Rong *et al.*, 2022, 2024). However, the presence of *K. aerogenes* in soil could be attributed to its ability to thrive in diverse conditions, in addition to the role of soil to act as potential reservoir for its spread.

For risk factors, positive *K. aerogenes* infection was observed significantly in patients aged 30-50 (11.86%) and >50 (14.63%) years more than <30 (0%) years; males (14.66%) more than females (7.41%); type 2 diabetes (14.17%) more than type 1 (6.25%); and glycemic uncontrolled (17.07%) more than glycemic controlled (8.2%) patients. Subsequently, the risk of *K. aerogenes* infection was elevated significantly in patients aged >50 years more than 30-50 years and <30 years; males more than females; type 2 diabetes more than type 1; and glycemic uncontrolled more than controlled patients. Although, no national or international studies have investigated the association of *K. aerogenes* infection to risk factors of patients with diabetic ulcers, several studies have been conducted to estimate the relationship between diabetic foot ulcers and multiple risk factors. In a previous study on 725 diabetic subjects, the risk of diabetic ulcers was greater in patient with mean age 65.9 years suggesting that foot ulcer and lower extremity vascular disease are related to a higher risk of death in diabetic subjects (Boyko *et al.*, 1996). In another study performed on 194 diabetic patients with new foot ulcers, the findings shown the majority of ulcers were neuropathic and present on the forefoot but without any effect for age, sex, duration/type of diabetes on outcome (Oyibo *et al.*, 2001). Orji *et al.* (2009) found that diabetic foot ulcers were increased significantly in males and females aged 30-49 years (24% and 25%, respectively) when compared to other age groups; 10-29 years (0%), 50-69 years (14% and 8%, respectively), and 70-89 years (18% and 105, respectively). In the later study, males were reported a higher rate of diabetic foot ulcers than females at age groups of 50-69 years (14% and 8%, respectively) and 70-89 years (18% and 10%, respectively). Costa *et al.* (2017) reported that the mean age of 654 diabetic patients was 63.1 years, and peripheral arterial disease was present in 24.5% concluding that diabetic foot ulcers is associated with old age and high mortality rates.

In a systemic review and meta-analysis study, difference in risk and sequelae of diabetic foot ulcers in males and females remains inconclusive; however almost studies have demonstrated that higher risk of disease was in males (Akinci *et al.*, 2011; Sayiner *et al.*, 2019; Gandhi *et al.*, 2020) but some studies found the absence of significance between females and males (Lin *et al.*, 2010; Tabur *et al.*, 2015).

Hangaard *et al.* (2019) mentioned that the screening information can be used for the risk assessment of foot ulcers among patients with type and type 2. In contrast, Schofield *et al.* (2021) detected that foot ulcer is an independent factor in type 1 and type 2 diabetes; however, authors recorded that the incidence rate of type 2 was higher (82.65%) than type 1 (17.35%) diabetes mellitus.

Xiang *et al.* (2019) investigated the role of glycemic control in healing of diabetic foot ulcers, and found that the wound healing rate was higher after the level of HbA1c was controlled between 7-8% but not at less than 7%. In a systemic review and meta-analysis of observational study, Lane *et al.* (2020) indicated that the higher HbA1c and fasting glucose are associated with increased amputation risk in patients with diabetic foot ulcers but neither HbA1c nor glucose levels were related with wound healing. Dutta *et al.* (2023) concluded that the early and intensive glycemic control in the first 4 weeks of treatment initiation is associated with greater healing of diabetic foot ulcers of initial ulcer area. Mustamu *et al.* (2024) found that the poor glycemic control with mean HbA1c of 8.3±1.4 and vascular complications such as peripheral artery disease and neuropathy were the most significant predictor of diabetic foot ulcer recurrence suggesting that controlling blood-sugar level, managing vascular issue, and promoting healthy behaviors can prevent diabetic foot ulcer recurrence.

5. CONCLUSION

This study provides the first molecular detection and phylogenetic characterization of *Klebsiella aerogenes* in diabetic foot ulcers (DFUs) in Iraq. The findings reveal a notable prevalence of *K. aerogenes* among DFU patients, with phylogenetic analysis confirming its genetic relatedness to clinically significant global strains. Important risk factors, including poor glycemic control and prolonged ulcer duration, were strongly associated with infection. These results highlight the clinical necessity of accurately distinguishing *K. aerogenes* from other Klebsiella species in diagnostic laboratories to ensure targeted antimicrobial therapy. From a public health perspective, the study underscores the urgent need for improved infection surveillance, antibiotic stewardship, and preventive strategies to reduce the burden of DFU-associated infections in Iraq.

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