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EIGHT NOVEL BACTERIAL SPECIES IDENTIFIED FROM CLINICAL INFECTIONS IN IRAQ: PHENOTYPIC, PHYLOGENETIC AND ANTIMICROBIAL RESISTANCE CHARACTERIZATION

Iman A. Chawshli[1*], Sawsan M. Sorchee[2]

[1] Department of Biology, College of Education, Salahaddin University-Erbil, Erbil, Kurdistan Region-Iraq. Email: iman.muhammad@su.edu.krd
[2] Department of Biology, College of Education, Salahaddin University-Erbil, Erbil, Kurdistan Region-Iraq. Email: Sawsan.ahdulla@su.edu.krd
***Corresponding author:** Iman A. Chawshli , Email: iman.muhammad@su.edu.krd

ABSTRACT

Bacterial diversification and their antimicrobial resistance (AMR) is a major clinical challenge worldwide. However, strain diversity and resistance patterns in regions with limited genomic surveillance remain critically under-characterized. This study aimed to identify and characterize unrecorded bacterial species circulating in clinical infections in Iraq. Swab samples were obtained from wounds-associated infections (i.e., burns, surgical, bed sores, blood, sputum and diabetic foot infections) in three hospitals of Erbil region, Iraq. Bacterial strains isolated from these samples underwent phenotypic identification, biochemical analyses and hemolytic activity testing. Phylogenetic characterization was performed using neighbor-joining approach based on 16S rRNA gene sequencing. AMR susceptibility was evaluated with a comprehensive set of antibiotic agents. A total of 84 clinical samples were obtained from 50 patients. Among the 21 identified species, eight were newly reported in Iraqi clinical samples. These include *Providencia huaxiensis*, *Pseudomonas beijingensis*, *Proteus columbae*, *Proteus faecis*, *Proteus myxofaciens*, *Escherichia albertii*, *Pseudescherichia vulneris*, and *Thiopseudomonas alkaliphila*. Importantly, majority of these species illustrated multidrug resistant phenotypes, with significant resistance to an extensive set of antibiotic agents (except for amikacin). This study provides the first comprehensive characterization- phenotypic, phylogenetic, and AMR- of eight novel bacterial species in Iraq, indicating a substantial and previously under-recognized diversity. This study may have important implications for bacterial surveillance and subsequent treatment.

KEYWORDS: Bacterial strains, PCR, GenBank, genomic diversity.

INTRODUCTION

Advanced molecular techniques such as whole-genome sequencing have demonstrated rapid genetic diversification in bacterial pathogens (Mustapha et al., 2022). Such strain-level genomic heterogeneity is primarily governed by the recombination, mutation, and horizontal gene transfer (Arnold, Huang and Hanage, 2022; Shikov et al., 2023). Moreover, the genomic diversification of bacterial strains triggers expanded and amplified antimicrobial resistance (AMR), virulence, and transmissibility (Mehrotra et al., 2023). Consequently, these genomic variations pose an increasing challenge to public health and the clinical management of infectious diseases. In this perspective, genomic monitoring of bacterial pathogens with strain-level accuracy has a direct clinical impact. Specifically, early identification of novel and divergent bacterial pathogens is critical for effective treatment and improved quality of life (Helmy et al., 2023).

Despite the direct clinical importance, there are large variations in genomic surveillance strategies and implementation across the globe (Oliveira Roster et al., 2024). In particular, global genomic databases (e.g., NCBI, PubMLST) are dominated by strain-level genomic data from high-income countries, while low- and middleincome countries remain underrepresented (Liu et al., 2025). To exemplify, data for bacterial isolates from the Middle Eastern countries, such as Iraq, remain limited in the international genomic repositories (Ateia et al., 2023). Such limited data potentially hinder detection and tracking of AMR trends and the identification of transmission pathways. Moreover, the limited bacterial characterization in these regions provides only a partial view of circulating pathogens and their susceptibility profiles.

Routine microbiological studies of samples collected from patients presenting to various clinics in Iraq have identified bacterial isolates with atypical phenotypic characteristics. More interestingly, the AMR profiles of these isolates were inconsistent with established resistance patterns of common bacterial strains in the region (Alaali and Bin Thani, 2020). These findings suggest divergence from known strains and the presence of previously unreported species. Consequently, this triggered additional investigation to correlate phylogenetic profiles with AMR determinants. Such species-level studies are of direct clinical importance, as they may influence both treatment efficacy and epidemiology patterns.

This study presents the first comprehensive characterization of eight previously unreported bacterial species, which were isolated from clinical infections in Iraq. Specifically, this study utilized phenotypic, biochemical, and molecular techniques to identify and characterize the unreported species, in tandem with an extensive investigation of AMR profiling. This study bridges a critical gap in global microbial surveillance by presenting region-specific data from an underrepresented setting, which is of significant importance for diagnostic refinement, epidemiologic tracking and antimicrobial stewardship. Overall, this study expands the clinical cohort of bacterial species in Iraq, and helps to understand global heterogeneity of bacterial pathogens and AMR dynamics.

MATERIALS AND METHODS

A. Sample collection and identification of pathogenic bacteria

During June-September 2024, eight previously unreported bacterial species were first isolated in Iraqi hospitals using a culturomics-based approach. Briefly, clinical samples were collected from three hospitals, including West Emergency Hospital, Erbil, Emergency Management Center, Erbil and Lalav Hospital, Erbil. Swab samples were obtained from patients presenting with burn and surgical wounds, bed sores, diabetic foot infections, blood, and sputum. Each sample was cultured on MacConkey, blood, nutrient, and mannitol salt agar, then aerobically incubated at 37°C for 24-48 hours. Colony formation was characterized based on morphology, pigmentation, hemolysis, and fermentation patterns (i.e., lactose or mannitol). Differential media was used to obtain pure cultures, which were preserved in 30% glycerol stocks at –80°C. Strains were identified using morphology, biochemical characteristics, and Gram staining (Atom Scientific, UK). The Vitek 2 Compact system (Biomerieux, USA) was used to confirm species-level identification. Specifically, a suspension of isolated colonies was prepared in physiological saline (turbidity: 0.50–0.63) using the Vitek 2 DensiChek device. Gram negative and Gram positive cards (bioMérieux) were also loaded into the Vitek 2 Compact system, and then incubated at 35– 37°C for 6–18 hours to identify the pathogens.

B. Molecular Identification

Genomic DNA was extracted from pure cultures using the Beta Bayern bacterial DNA extraction kit (Beta Bayern GmbH, Germany) and eluted in 100 µL buffer. Extracts were stored at –20°C until PCR analysis. DNA concentration and purity were assessed with a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, USA). The 16S rRNA gene was amplified in 50 µL reaction mixture containing 25 µL of 2×Taq DNA Polymerase Master Mix (AMPLIQON A/S, Stenhuggervej 22), 2 µL (10 pmol) of each primer 16S-F (5'-CGTTGACTGCCGGTGACAAAC-3') and 16S-R (5'-CTTTTCCTCCGCTTATTGATATG-3'), 3 µL of template DNA (50 ng/µL), and 18 µL of DNase-free water. The primers were designed using the Primer3Plus software (GenBank accession no. OL336429.1). The amplification was carried out in a Bioresearch PTC-200 Gradient thermocycler with initial denaturation at 95°C for 5 min, 35 cycles of denaturation at 95°C for 40 s, annealing at 58°C for 40 s, and extension at 72°C for 1 min; followed by a final extension at 72°C for 10 min. Amplified products were subjected to electrophoresis using a 1.2% agarose gel (GeNet Bio, Korea) containing 1x GelRed DNA stain and purified using a PCR purification kit (Qiagen, Germany).

Sequencing of the partial 16S rRNA gene was performed using ABI Prism Terminator Sequencing Kit (Applied Biosystems) at Macrogen Molecular Company, Korea. Chromatograms were edited, and base calls were verified using the Finch TV program software. Finally, the gene sequences were applied to the Basic Local Alignment Search Tool (BLAST) to compare against the existing bacterial sequences in the GenBank, NCBI database, and to characterize the phylogenetically closest species.

C. Biochemical testing

Pure cultures grown on non-selective agar were used to prepare standardized inocula. Biochemical assays were performed according to standard microbiological protocols to metabolically characterize the bacterial isolates. Briefly, Indole production was assessed using Kovac's reagent after growth in tryptone broth. Citrate utilization was evaluated on Simmons citrate agar. Oxidase activity was tested using oxidase reagent strips, while urease activity was determined using Christensen's urea agar. Triple Sugar Iron (TSI) agar slants were used to examine glucose, lactose, and sucrose fermentation, along with gas and H_2S production; slant and butt reactions were recorded after incubation (Chauhan and Jindal, 2020).

D. Hemolysis testing

Hemolytic characterization was performed on 5% sheep blood agar (Oxoid, UK). Briefly, overnight cultures were streaked onto the agar surface and incubated at 37°C for 24 h under aerobic conditions. Following incubation, colonies were examined for hemolysis patterns and classified as β-hemolysis (complete lysis with clear zones), α-hemolysis (partial lysis with greenish discoloration), or γ-hemolysis (absence of lysis) (Ridder et al., 2021).

E. Motility assay

To examine surface motility, a single bacterial colony was inoculated in 1-3 ml of nutrient broth medium (NB) (Scharlau, Spain) and incubated at 37°C for 16-18 h with shaking at 180 rpm. Moreover, 5 µL of each isolate was inoculated in the center of solid media (blood and nutrient agar) and on a motility agar plate (NB containing 0.35% agar), followed by an incubation at 37°C for 18-24 hours. The motility rate was calculated as the diameter of resulting growth pattern over time. Also, the motility test was done inside the screw tube containing motility agar. Each isolate was stabbed with sterile needle in the center of motility agar tube at half of the medium, then incubated at 37°C for 24-48 hours. Positive motility was indicated by diffuse growth from the stab line, while the negative motility test was indicated by growth confined to the inoculation line (Clemmer, Bonomo and Rather, 2011; Juliane et al., 2017).

F. Antibiotic susceptibility testing

Susceptibility of the bacterial strains was assessed against a set of 34 antibiotic agents, including amikacin, gentamicin, amoxicillin/clavulanic acid, piperacillin/tazobactam, cefuroxime, ceftriaxone, ceftazidime, imipenem, meropenem, trimethoprim/sulfamethoxazole, ciprofloxacin, nitrofurantoin, fosfomycin, cefazolin, cefepime, cefuroxime axetil, ertapenem, cefotaxime, ampicillin/sulbactam, ceftolozane/tazobactam, colistin, tigecycline, benzylpenicillin, clindamycin, erythromycin, tetracycline, teicoplanin, vancomycin, rifampicin, levofloxacin, linezolid, moxifloxacin, oxacillin, and tobramycin. The Vitek-2 system with Gram-Negative susceptibility cards (AST-N417 and AST-N419) and Gram-Positive susceptibility cards (AST-P580: Biomerieux, USA) was used for this purpose. The susceptibility findings were defined as sensitive, intermediate, or resistant based on the criteria of Clinical and Laboratory Standards Institute (CLSI) (Koumaki et al., 2024).

G. Statistical analysis

Statistical analyses were performed using GraphPad Prism version 8. Heatmaps were used for visualization of interspecies variations and resistance trends within the dataset.

RESULTS

A. Study population and bacterial isolates

A total of 84 clinical samples were obtained from 50 patients (males: females = 1.63, age range: 1-60 years, Supplementary Table SI and Table SII). These samples were obtained from burn wounds (38: 45.3%), surgical wounds (10: 11.75%), bedsores (05: 5.95%), diabetic foot infections (06: 7.14%), blood (06: 7.14%), and sputum samples (19: 22.62%). All samples produced positive bacterial cultures, demonstrating either monobacterial (14: 28%) or polybacterial growth (36: 72%). In total, 21 distinct bacterial species were identified, including eight unreported species from Iraq. Table I shows the analysis of all 21 species, which demonstrates a heterogeneous distribution of the bacterial isolates across the clinical samples. Among the 21 species identified, the predominant bacterial pathogens were *Acinetobacter baumannii* (21: 25%), *Pseudomonas aeruginosa* (10: 11.9%), and *Staphylococcus aureus* (9: 10.71%). Overall, 61.9% of *Acinetobacter baumannii* and 40% of *P. aeruginosa* were found in burn wounds. It is important to note that eight bacterial species were recorded for the first time in Iraqi clinical samples. These species included *Providencia huaxiensis*, *Pseudomonas beijingensis*, *Proteus columbae*, *Proteus faecis*, *Proteus myxofaciens*, *Escherichia albertii*, *Pseudescherichia vulneris*, *Thiopseudomonas alkaliphila*; these unreported species are shown in bold font in Table I.

Table I Distribution of bacterial species (expressed in percent) across all clinical samples. The unreported strains are shown in bold font.

Bacterial strain	Burn wound	Surgical wound	Bedsore	Diabetic foot infection	Blood	Sputum
Acinetobacter baumannii	61.9	14.2	0	4.7	9.6	9.6
Providencia stuartii	66.6	16.7	0	0	0	16.7
Providencia huaxiensis	0	100	0	0	0	0
Pseudomonas aeruginosa	40	10	10	0	10	30
Pseudomonas beijingensis	50	50	0	0	0	0
Proteus mirabilis	33.3	0	0	33.3	0	33.3
Proteus hauseri	100	0	0	0	0	0
Proteus columbae	0	0	0	100	0	0
Proteus faecis	0	0	50	0	50	0
Proteus alimentorum	50	0	50	0	0	0
Proteus myxofaciens	0	0	0	50	0	50
Klebsiella pneumoniae	33.3	0	0	0	0	66.7
Klebsiella variicola	33.3	0	0	0	0	66.7

Klebsiella quasipneumoniae	0	0	25	50	0	25
Escherichia albertii	50	0	0	0	0	50
Pseudescherichia vulneris	50	25	0	0	0	25
Serratia fonticola	50	0	50	0	0	0
Thiopseudomonas alkaliphila	0	0	0	0	0	100
Enterobacter kobei	0	100	0	0	0	0
Staphylococcus aureus	66.7	0	0	0	11.1	22.2
Lysinibacillus sphaericus	33.3	33.3	0	0	33.3	0

B. Molecular identification and phylogenetic analysis

PCR studies of the bacterial 16S rRNA gene yielded a clear and consistent 372-bp band across all eight unreported isolates (Fig. 1). This corresponded to the expected amplicon size of the targeted 16S rRNA gene fragment. Moreover, no amplification was observed in negative controls (NC), indicating high primer specificity and efficient amplification.

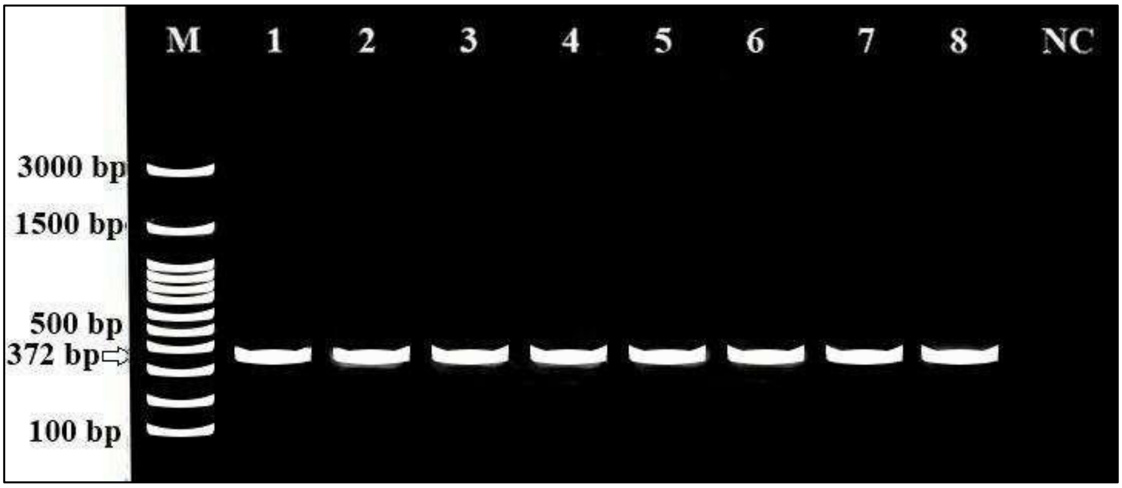


Fig. 1. PCR amplification of the partial 16S rRNA gene from the unreported bacterial strains. M: Ladder (3000-100 bp), NC: negative control, lanes: 1-8 gene bands with 372 bp amplification.

Table II presents GenBank accession numbers of the eight unreported bacterial isolates. Results from sequence analysis showed 99-100% similarity for these bacterial strains with the reference sequence of GenBank. The 16S rRNA sequence data for the bacterial species were submitted to NCBI GenBank.

Table II Accession numbers of the eight unreported bacterial species identified according to the BLAST of GenBank NCBI of the partial 16S rRNA gene.

Sr. No.	GenBank accession number (16S rRNA)	Genus and species of bacterial strain	Accession of number submitted sequence	Query cover (percent)	Identic number (percent)
1	CP145931	*Providencia huaxiensis*	PQ458503	100	100
2	NR_174258	*Providencia huaxiensis*		100	100
3	CP117451	*Pseudomonas beijingensis*	PQ657275	100	100
4	NR_197706	*Pseudomonas beijingensis*		100	99.66
5	CP043925	*Proteus columbae*	PQ458499	99	100
6	MZ169441	*Proteus columbae*		99	100
7	PQ45851	*Proteus faecis*	PQ458516	100	100
8	OQ406241	*Proteus faecis*		100	100
9	MF574017	*Proteus myxofaciens*	PQ458496	100	100
10	NR_043999	*Proteus myxofaciens*		100	100

11	AP014856	*Escherichia albertii*	PQ458505	100	100
12	HM194885	*Escherichia albertii*		100	100
13	CP086374	*Pseudescherichia vulneris*	PQ458492	100	100
14	LR607338	*Pseudescherichia vulneris*		100	100
15	CP012365	*Thiopseudomonas alkaliphila*	PQ517188	100	100
16	CP012360	*Thiopseudomonas alkaliphile*		100	100
17	CP012361	*Thiopseudomonas alkaliphila*		100	100

Fig. 2 shows the phylogenetic tree analysis of the unrecorded species based on 16S rRNA gene sequences. Briefly, the phylogenetic tree was constructed using the neighbor-joining method. The divergence similarity data of DNA sequences was used to group the species into clades corresponding to their genera. Thus, the taxonomic classification of unrecorded species was confirmed by their clustering with reference sequences (i.e., GenBank) of closest phylogenetic relatives.

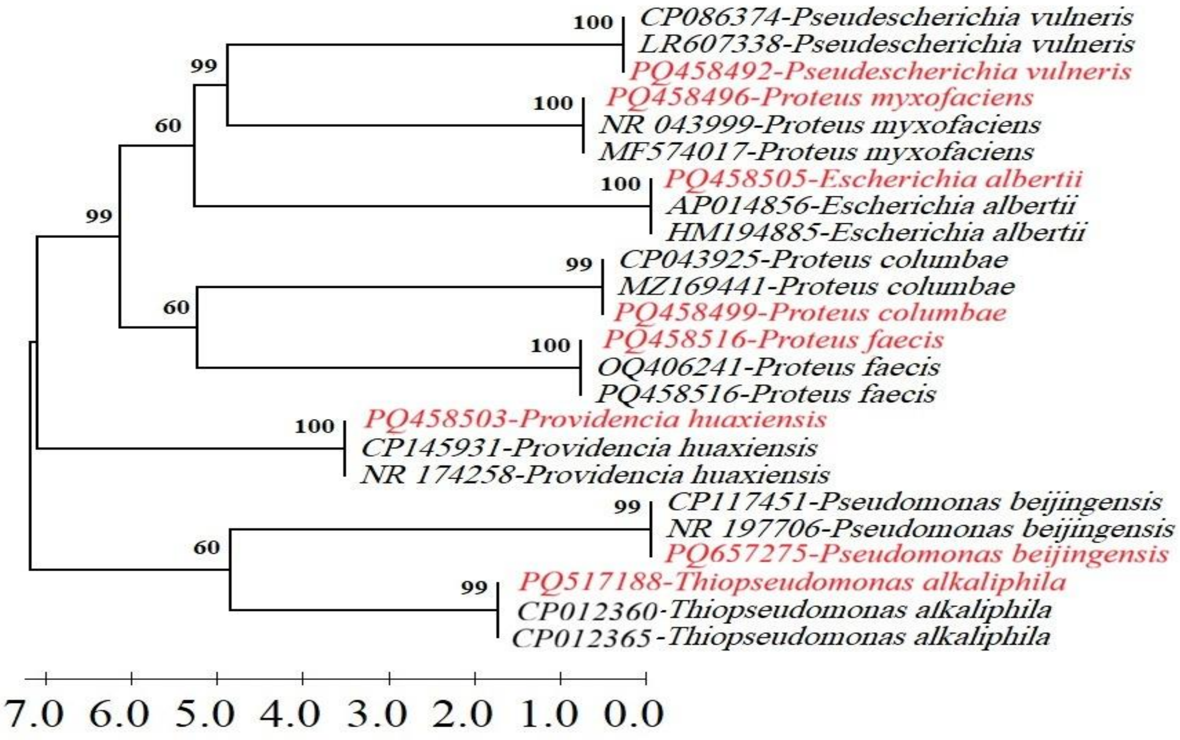
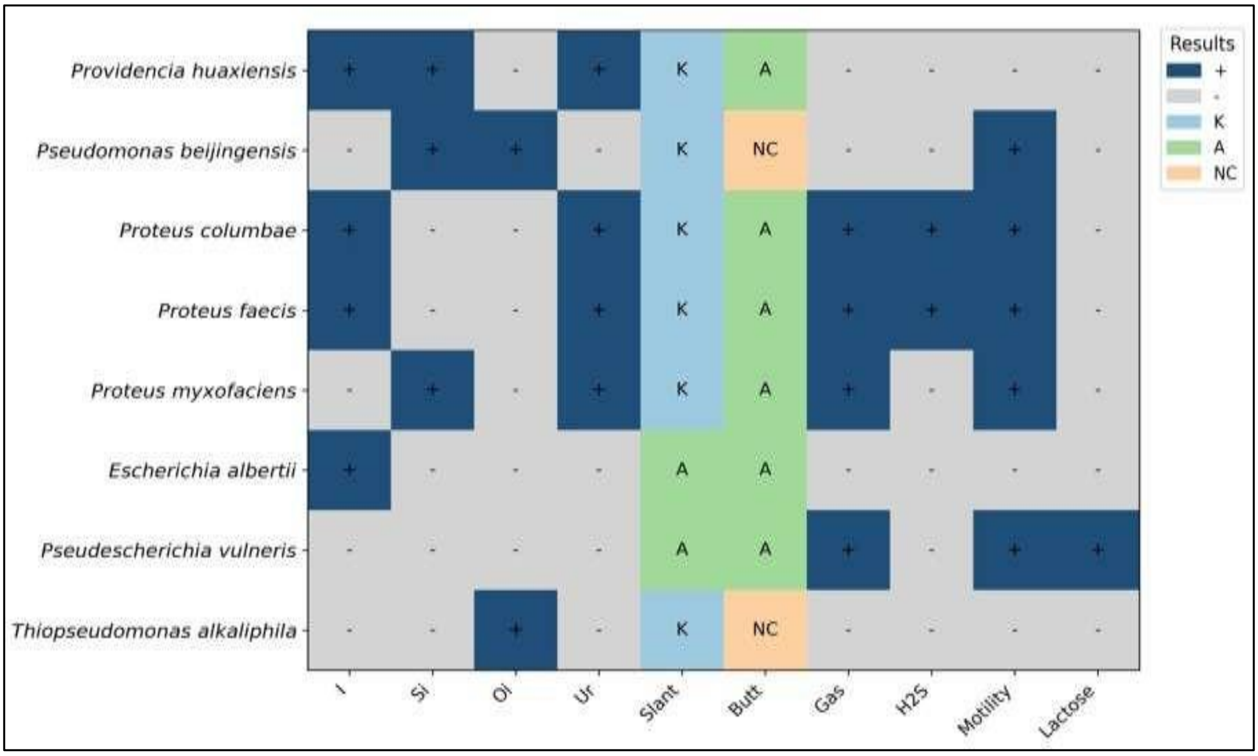


Fig. 2. Phylogenetic tree constructed using the neighbor-joining method based on the 16S rRNA gene sequences of the eight unreported bacterial species isolated from Iraqi clinical samples. The unreported strains are shown in red pseudocolor.

C. Biochemical and motility analyses

Fig. 3 shows the biochemical profiling of the newly recorded bacterial isolates in Iraqi clinical samples. Specifically, the biochemical characterization included indole, citrate, oxidase, urease, triple sugar iron (TSI), gas formation, H_2S production, motility, and lactose fermentation. The results demonstrated interspecies variability across these metabolic metrics. In particular, citrate utilization was positive in *Providencia huaxiensis*, *Pseudomonas beijingensis*, and *Proteus myxofaciens*, whereas indole production and urease activity varied among the genera. *Providencia huaxiensis* and *Proteus* spp. exhibited distinctive alkaline slant/acid butt patterns in the TSI reaction. In contrast, *Escherichia albertii* and *Pseudescherichia vulneris* showed uniform acid slant/acid butt. Moreover, all of unrecorded bacterial species exhibited negative oxidase reactions except *Pseudomonas beijingensis* and *Thiopseudomonas alkaliphila* as well as lactose fermentation was restricted mainly to *Pseudescherichia vulneris*. Motility tests revealed variable motile behavior among *Proteus* and *Pseudomonas* species.



	I	Si	Oi	Ur	Slant	Butt	Gas	H2S	Motility	Lactose
Providencia huaxiensis	+	+	-	+	K	A	-	-	-	-
Pseudomonas beijingensis	-	+	+	-	K	NC	-	-	+	-
Proteus columbae	+	-	-	+	K	A	+	+	+	-
Proteus faecis	+	-	-	+	K	A	+	+	+	-
Proteus myxofaciens	-	+	-	+	K	A	+	-	+	-
Escherichia albertii	+	-	-	-	A	A	-	-	-	-
Pseudescherichia vulneris	-	-	-	-	A	A	+	-	+	+
Thiopseudomonas alkaliphila	-	-	+	-	K	NC	-	-	-	-

Fig. 3. Heatmap analysis for biochemical tests of unrecorded Gram-negative isolates in Iraq. In which (I) Indole, (Si) Simmon citrate, (Oi) Oxidase, (Ur), Urease test, and (NC) No change.

Motility assay demonstrated distinct swarming and spreading patterns among the new recorded bacterial isolates (Fig. 4). Briefly, *P. columbae, P. myxofaciens, P. beijingensis*, and *P. vulneris* expressed a characteristic concentric or dendritic swarming rings on culture plate, although the intensity and morphology of the swarming fronts differed among species. *P. faecis* demonstrated a pronounced multi-zone swarming expansion with irregular branching edges. In motility medium of screw tubes, all isolates displayed diffuse turbidity extending away from the stab line, indicative of positive motility.

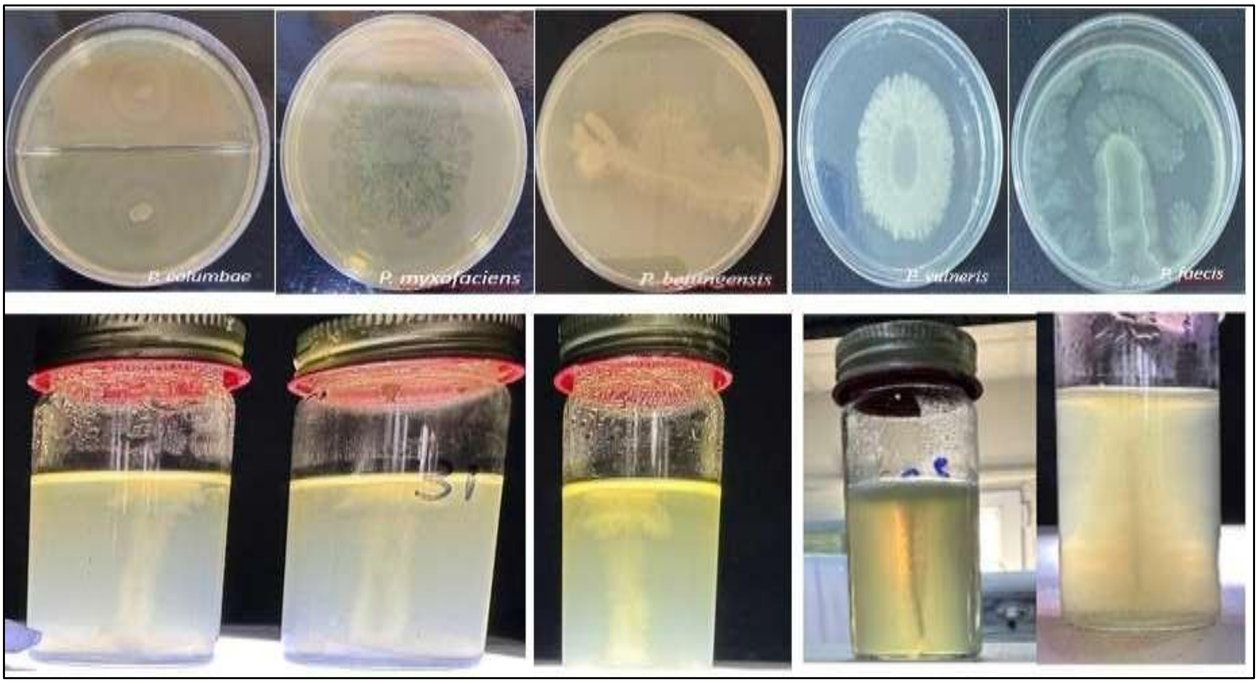


Fig. 4. Motility test patterns and colony morphology of selected newly recorded bacteria cultured in plates and tubes with semisolid media.

D. Hemolytic test activity

Hemolytic activity showed a heterogeneous behavior in erythrocyte lysing capacity among the clinical isolates, as summarized in Table III). Among the eight unrecorded species, the γ-hemolysis was substantially predominant than the β-hemolysis. In particular, only *Pseudomonas beijingensis* illustrated β-hemolysis.

Table III Hemolytic activity of the newly recorded bacterial species

Sr. no	Species	Sample Source	Hemolysis Type	Colony Morphology on NA & MHA
1	*Providencia huaxiensis*	Surgical wound	γ	White circular colony
2	*Pseudomonas beijingensis*	Burn wound Surgical wound	β	Pale yellow round smooth colony
3	*Proteus columbae*	Diabetic foot infection	γ	Translucent, Circular colony
4	*Proteus faecis*	Bedsore blood	γ	Pale yellow, Swarming growth, thin spreading film
5	*Proteus myxofaciens*	Diabetic foot infection Sputum	γ	Smooth, translucent colony
6	*Escherichia albertii*	Burn wound sputum	γ	colorless, translucent, smooth, circular colony
7	*Pseudescherichia vulneris*	2 Burn wound Surgical wound Sputum	γ	Smooth, shiny, small-medium round colony, pale yellow/white
8	*Thiopseudomonas alkaliphila*	2 sputum	γ	Light brown, circular colony

E. Antibiotic susceptibility

Although antibiotic susceptibility testing was performed for all 21 pathogens, detailed results are presented for only the newly identified species in Figure 5. In the Gram-negative cohort, several newly recorded taxa from Iraq showed distinctive resistance signatures. Specifically, *Providencia huaxiensis* was completely resistant to carbapenems, cephalosporins, ciprofloxacin, gentamicin, and ampicillin/sulbactam. Likewise, *P. beijingensis* showed complete resistance to imipenem, ceftazidime, gentamicin, and piperacillin/tazobactam. *P. faecis* exhibited complete resistance across all utilized antimicrobial agents except amikacin, whereas *P. alimentorum* had intermediate resistance of 50% to piperacillin/tazobactam and ampicillin/sulbactam. Moreover, *P.* columbae was completely resistant while *P. myxofacience* was partially (50%) resistant to tigecycline only. In addition, *E. albertii* showed high resistance to all tested antimicrobials except amikacin and nitrofurantoin, while *Pseudescherichia vulneris* displayed complete resistance to imipenem, meropenem, ciprofloxacin, cefepime, cefotaxime, gentamicin and partial resistance to β-lactam/ βLI 75% and ceftazidime 50%. Finally, *T. alkaliphila* was completely resistant to ceftazidime/avibactam, ceftolozane/tazobactam, ceftazidime, as summarized in Fig 5.

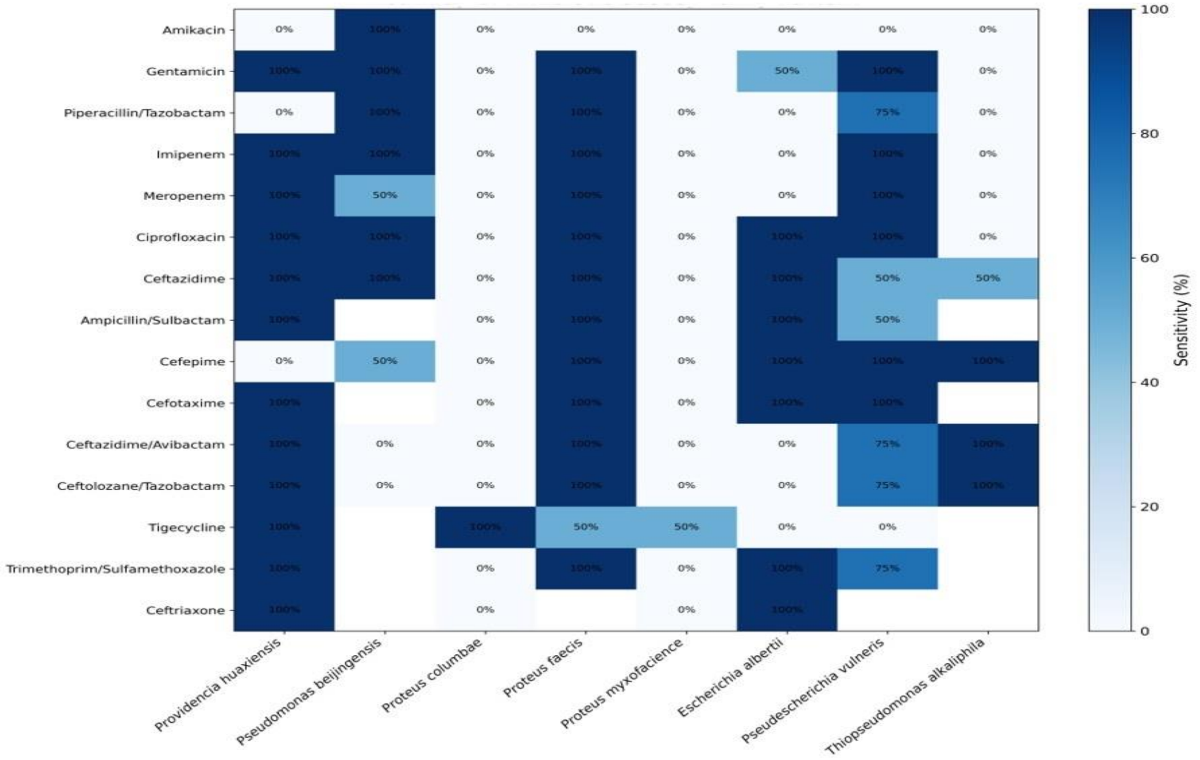


Fig. 5. Heatmap showing percentage of antibiotic susceptibility for the unreported bacterial strains. The dark blue, light blue and white pseudocolors represent complete (100%), partial (75%), (50%), and no (0%) resistance while clear one indicates not tested antibacterial agent.

DISCUSSION

In this study, eight previously unreported bacterial species were identified in clinical samples from three different hospitals of Iraq. These species were isolated from patients presenting with different infection sources, including surgical wounds, burn wounds, bedsores, diabetic foot infections blood and sputum. These pathogens were characterized and their distinction confirmed with phenotypic-, molecular-, biochemical-analyses and AMR susceptibility. Overall, this study demonstrated the pathogenic heterogeneity and eight unrecorded species in Iraqi clinics, and enhances Middle East representation in international databases.

Identification of the eight new strains was validated with molecular studies (i.e., partial 16S rRNA gene amplification) and phylogenetic analysis (i.e., the neighbor-joining approach for tree formation). Identification of these new species indicated that the pathogenic diversity in Iraq is broader than the prevailing diagnostic framework. This emphasize that although the present biochemical methods used for microbial surveillance are valuable, these approaches may not be sufficient to accurately identify divergent or atypical strains. Consequently, it seems of significant benefit to integrate molecular and phenotypic techniques in diagnostic clinics.

This study revealed site-specific distribution patterns of the bacterial isolates. For instance, we isolated the *Providencia huaxiensis* from the surgical wounds. Previously, this strain has been sparsely reported from human clinical substrates (e.g., initially isolated from a rectal swab) (Hu et al., 2019). *Providencia huaxiensis* has also been recovered from incision infection and intracranial infection (Yang et al., 2024). The same strain has been found in surgical wound in this study. *Pseudomonas beijingensis*, observed in burn and surgical wounds herein, was reported in colonize in plant samples (Liao et al., 2024). *Proteus columbae* was isolated from diabetic foot infection, which, to the best of our knowledge, is described for the first time globally. Likewise, the *Proteus faecis* is frequently reported in fresh water finless porpoise (McLaughlin et al., 2024). However, this study found *Proteus faecis* in samples obtained from infections caused by bedsore and blood. It may be noted that this strain has been reported in flesh specimen obtained from pigeon (Dai et al., 2018). Moreover, *Thiopseudomonas alkaliphila* was recovered from sputum samples in this study for the first time globally (Drobish et al., 2016; Burcham, 2024). Studies have linked detection of this novel strain in diverse sources, including industrial fermenters (Ramalingegowda et al., 2026), animal guts (Guo et al., 2018), human tissues (Burcham et al., 2024), and decomposition environments (Cobaugh, Schaeffer and DeBruyn, 2015; Keenan et al., 2018). Although such studies indicate a diverse ecological role of *Thiopseudomonas alkaliphila*, a large knowledge gap still exists regarding its functions. Importantly, *Proteus alimentorum* was previously reported from Iraq, but not submitted to NCBI GenBank (Turkei and Al-dulaimi, 2024). This study found *Proteus alimentorum* in burn wounds and bedsores. However, it was also documented in a patient with peritoneal cancer (Mori et al., 2023).

Regarding the previously reported species, burn wounds dominantly harbored Acinetobacter baumannii (61.9%) and *Pseudomonas aeruginosa* (40%). This is consistent with global epidemiology of burn wounds. Moreover, past studies from Baghdad, Iraq also supports these study findings (Al-Azzawi and Alkalifawi, 2023). Specifically, these studies reported fairly prominent isolation of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* from burn wounds. In addition, several studies have documented *Acinetobacter baumannii* as a dominant pathogen in burn wounds (Roy et al., 2024). The most common strains in burn infection were *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in Morocco (Hamzaoui et al., 2020), *Pseudomonas aeruginosa* and *Staphylococcus aureus* in Iran (Ekrami and Kalantar, 2007), Saudi Arabia (Alharbi and Zayed, 2014), and Egypt (Ahmed et al., 2023). Such patterns likely reflect differences in pathogenic potential, environmental adaptability, and clinical relevance across infection types. The newly recorded taxa from Iraq showed distinctive resistance signatures. Specifically, the data indicated that *Proteus faecis* and *Providencia huaxiensis* exhibited complete (100%) resistance to 12 antibiotic agents, while the *Escherichia albertii* to 07 and *Pseudomonas beijingensis* to 06 antibiotic agents. Such findings may have direct clinical applications and may indicate the need for strict stewardship. The prominence of carbapenem and cephalosporin resistance in this study accords with recent global and Iraqi reports (Mori et al., 2023; Al-fahad et al., 2024; Liao et al., 2024). Alternatively, amikacin retained complete activity against all newly identified species, except *Pseudomonas beijingensis*. Such findings are of significant clinical concern and underscore the need for strict antimicrobial stewardship. They also highlight the importance of rapid detection of carbapenemase and ESBL production, along with timely MIC-guided therapeutic escalation to ensure appropriate management of these strains.

This study was limited by a relatively small sample size obtained from selected clinics. This may hinder a complete understanding of the clinical distribution and diversity of unreported species on a national level. Moreover, wholegenome sequencing was not performed, which restricted identification of gene responsible for virulence and antibiotic resistance. Finally, although susceptibility profiles of the unreported strains were explored, the exact mechanism governing the resistance was not studied. Despite these limitations, this study highlighted the importance of systematic microbiological and molecular surveillance in regions that are underrepresented in the global databases, including Iraq.

CONCLUSION

This study identified and characterized eight previously unrecorded bacterial species in Iraq. These species were isolated from wound-associated infections and included *Providencia huaxiensis*, *Pseudomonas beijingensis*, *Proteus columbae*, *Proteus faecis, Proteus myxofaciens, Escherichia albertii*, *Pseudescherichia vulneris*, and *Thiopseudomonas alkaliphila*. The phenotypic, biochemical, and molecular analyses demonstrated substantial taxanomic and functional diversity in bacterial strains. Moreover, susceptibility of these unrecorded strains to an extensive set of antibiotic agents revealed significant resistance (except for amikacin). These findings indicate the diversity of bacterial strains circulating in Iraqi clinics and the underrepresentation of the regional genomics in global databases.

Declarations

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Author contribution: SM designed the research; IA conducted the research, analyzed data, and wrote the paper; SM edited the paper; IA and SM had primary responsibility for final content. Both authors read and approved the final manuscript.

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