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Detection of some virulence genes (*Esal* and *OmpA*) in *Pantoea* spp. isolated from different clinical specimens from patients referred to hospitals in Mosul (Iraq)

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Pantoea spp. (previously *Enterobacter* spp. and *Erwinia* spp.) are Gram negative aerobic or facultatively anaerobic bacilli in the family Erwiniaceae. They are opportunistic pathogens that cause different human infections. Our study aims to investigate the existence of *Pantoea* spp. isolated from clinical specimens of patients in hospitals of Mosul city, study their antibiotic resistance and detect some virulence genes of these isolates. For the identification of *Pantoea* spp., we used Analytical Profile Index 20 (API 20). Extraction DNA from isolates was subjected to 16S rRNA amplification by PCR. We detected the existence of factors responsible for pathogenesis (*Esal*, *OmpA*) genes. The highest number of *Pantoea* spp. in clinical samples was found in wound swabs followed by urine and ear swabs, 5.00%, 1.88% and 1.33% respectively. *Pantoea* spp. infection prevalence was higher in males than females. The present study explained that *Pantoea* spp. were more sensitive to meropenem and cefotaxime than other antibiotics. The molecular diagnosis of all isolates (fourteen) was confirmed by 16S rRNA. Seven isolates were studied for investigation of virulence factors, results showed that 7/7 (100%) isolates contained the virulence genes (*Esal*, *OmpA*), which participate to increase their pathogenicity. *Pantoea* spp. was isolated from different clinical specimens, so it may be the cause of various infections in humans. The PCR technique detected the existence of genes which are responsible for virulence (*Esal* and *ompA*), these genes are important in causing bacterial infections. Genetic variation was examined, showing multiple mutation of (16S rRNA, *Esal*) genes, and point mutation of the *OmpA* gene.

Keywords: *Pantoea* spp.; clinical specimens; virulence genes; *Esal*; *OmpA*.

Introduction

Pantoea spp. (previously *Enterobacter* spp, *Erwinia* spp.) are Gram-negative aerobic or facultatively anaerobic bacilli, usually motile and vary in gas production and lactose fermentation (Sulja et al., 2022). Genus *Pantoea* belongs to the Family: Erwiniaceae, Order: Enterobacterales. Class: Gammaproteobacteria, Phylum: Pseudomonadota, Domain: Bacteria.

Pantoea is classified into about twenty species, which can exist in soil, water and plants (Kini et al., 2021; Doni et al., 2021; Lorenzi et al., 2022; Lv et al., 2022; Yao et al., 2023). They can be either commensals or opportunistic pathogens (Hani et al., 2023), and have been isolated from insects, animals and human wounds (Olmos-Alpiste et al., 2022; Sato et al., 2023; Susanto et al., 2023). They can cause other human infections like soft tissue or bone / joint infections following penetrating trauma by vegetation (Koester et al., 2020; Koutserimpas et al., 2022), bacteremia by the contamination of intravenous fluid (Asai et al., 2019; Oliveira et al., 2019; Cobo et al., 2022), total parenteral nutrition, blood products, and anesthetic agents (Yablon et al., 2017; Borrego et al., 2020; Susanto et al., 2023). *Pantoea* spp. also cause skin infections, and urinary tract infections acquired from communities, and others, but at low rates compared with infections caused by other Gram-negative rod bacteria as *Proteus* spp., *Klebsiella* spp., and *E. coli* (Okwundu & Mercer, 2019; Fazaa & Darweesh, 2020; Zahraa et al., 2023; Mhuireach et al., 2023, Kahn et al., 2024). This is because the genus *Pantoea* is considered an opportunistic organism, of low virulence, little intrinsic invasiveness and low degree of toxicity, but also it can cause infection in individuals with healthy immune systems. And it is fatal for postoperative, infants, and immunocompromised patients (Casale et al., 2023; Kolle et al., 2025). Species of this genus may cause infections acquired from hospital such as respiratory tract infection or urinary tract infection and other infections. The main transmission route of this bacteria is contact directly or indirectly with persons or contaminated objects. *Pantoea agglomerans* is the most common species of *Pantoea* genus which is isolated in humans. (Al-Kidsawey et al., 2020; Mirtella et al., 2021; Shu et al., 2022).

In the recent years, the 16S rRNA sequence considered an important tool for the classification of *Pantoea* genus. However, it has low accuracy at the species level (Hameed Shahab et al., 2023).

There are many factors which play an important role in bacterial pathogenicity and antibiotic resistance such as the bacterial outer membrane, which represents a non-permeable membrane protecting them against the environment, bile salts and antibiotics. This membrane has a group of outer-membrane proteins (OMPs), named porins, by which most nutrients pass through them. The outer membrane contains a group of proteins related genetically and surface-exposed, which exist in high number smainly in pathogenic Gram negative bacteria. *OmpA* proteins have essential functions for invasion, adhesion, intracellular survival, escape from defenses of the host and as stimulators of pro-inflammatory cytokine production. These pathogenic roles are most commonly related to central nervous system, urogenital and respiratory diseases (Vergalli et al., 2020).

Efflux pumps are important defense mechanisms for *Pantoea* spp. that support their ability to cause disease and cause their resistance to antibiotics. These efflux pumps prevent exposure of bacteria to antibiotics. In addition the bacterial outer membrane is non permeable to large molecules of antimicrobial drugs (Du et al., 2018; Kajdacs, 2019). There are many virulence genes which are responsible for pathogenicity of *Pantoea* spp. such as the *OmpA*, *Esal* genes. The *Esal* gene encodes important proteins for quorum sensing and controls the production of the capsular polysaccharide by *Pantoea* spp. Exopolysaccharide plays an essential role in the adherence and virulence of bacteria (Hasson et al., 2018). Our research aims to detect the existence of *Pantoea* species in different clinical specimens and recognize their antibiotic susceptibility and detect some virulence genes of *Pantoea* spp.

Materials and methods

We conducted a cross-sectional study for investigating the prevalence of *Pantoea* spp. in different clinical specimens from patients referred to hospitals in Mosul city. We aimed to detect some of their virulence genes.

The number of clinical specimens were 435, which were collected from patients referred to Al-Salam, Al-Jumhori and Ibn Sina hospitals during the period 15/2/2023 to 15/10/2023. The following specimens were included: urine (160), ear pus (75), wound pus (200).

We cultured the specimens on (MacConkey & blood) agar, incubated aerobically at 37 ˚C, 24 hour. We examined the isolates under microscope by using Gram stain to study their properties as form and Gram reaction. We also studied the macroscopic characteristics of bacterial colonies (Procop et al., 2016). Identification was performed using the API 20E according to the manufacturer's instructions (bioMérieux). The inoculated strips were incubated at 37 °C for 24 hour. Results were interpreted by using the conventional methods. (Published instructions by BioMérieux in Appendix 1 for API 20 E).

This test was done by using Muller Hinton agar plate according to the method of Kirby-Bauer disc diffusion using Clinical and Laboratory Standards Institute (CLSI) as guidelines. The antibiotics usied in this study were: Cefotaxime (CTX) 30 µg, Ciprofloxacin (CIP) 5 µg, Chloramphenicol (C) 30 µg, Gentamicin (GEN) 10 µg, Tetracycline (TET) 30 µg, Amikacin (AMK) 30 µg, Meropenem (MEM) 10 µg, Trimethoprim/Sulfamethoxazole (SXT) 25 µg, Tobramycin (TOB) 10 µg, Clindamycin (CC) 2 µg, Cefalexin (LEX) 30 µg, Nalidixic acid (NA) 30 µg, and Nitrofurantoin (NIT) 300 µg.

The suspension of bacteria was prepared by inoculating five isolated colonies from brain heart infusion agar into five ml broth of tryptic soy and incubated at 37 °C, 2 h. Turbidity of this suspension was determined by comparison with a McFarland tube standard of 0.5. The suspension was spread on Mueller Hinton plates and antibiotic discs were placed on it and incubated at 37 °C, 24 h. Sensitivity and resistance to antibiotics were measured according to CLSI.

The DNA was extracted of seven isolates of the *Pantoea* spp. by using the instructions of the commercially manufactured kit prepared by the company (intron biotechnology/Korea). DNA extracted was measured at 260/280 nm by the nanodrop device (Kim et al., 2009).

Gene 16S rRNA primers were used for the diagnosis of *Pantoea* spp. This study investigated the existence of genes responsible for pathogenesis (*Esal*, *OmpA*) in seven isolates of *Pantoea* spp. by PCR amplification using a primer set for these genes. All primers were designed using NCBI – Gen bank and primer 3 plus design. These primers were prepared by Bioneer Co. in Korea (Table 1). Specific interaction mixture for gene diagnosis was explained in Table 2. The PCR optimum conditions for genes are showed in Table 3.

PCR products were sequenced by Macrogen DNA sequencing (Korea) by Sanger method using instruments: (Genetic Analyzer System ABI-310). Local sequences were aligned with reference sequences using the program of Basic Local Alignment Search Tool (BLAST) to identify them and the sample was registered to Gen Bank (ID) (Tamura et al., 2021). We determined the similar sequences for the genes in NCBI, then multiplied the sequence alignment and designed a phylogenetic tree by Neighbor-Join and BioNJ algorithms using the Maximum Composite Likelihood (MCL), MEGA11 program (Patakova et al., 2024, Legese et al., 2024).

Table 1
Primer sequences used in this study

Gene	Primer	Sequence	T_m (°C)	GC (%)	Product size	Reference
	Forward	5'– CAACGCGAAGAACCTTACCT– 3'	55.1	50.0	455	
16 s rRNA	Reverse	5'–CACTCCCATGGTGTGACG – 3'	55.8	61.1	base pair	28
	F	5'– ACCGAGTCCAGCCGTTTT– 3'	57.1	55.6	301	
Esal	R	5'– AGCCCGTACGTGCAACAATA – 3'	57.0	50.0	base pair	28
	F	5'–CGCATAGCACTCAAGTTTCTCC – 3'	56.0	50.0	202	
OmpA	R	5'–CATAAACAGATTGACCGAAACG –3'	52.1	40.9	base pair	37

Table 2
Specific interaction mixture for gene diagnosis

Component	Concentration
PCR Taq Pre Mix	5 µL
(primer) forward	1 µL (10 picomols/µL)
(primer) reverse	1 µL (10 picomols/µL)
(DNA)	1.5 µL
Distilled water	16.5 µL
Total volume	25 µL

Data was summarized and analyzed by statistical package SPSS ver. 26. The descriptive method was used to summarize the data and it was presented as percentages and by pie chart. A Z test for comparison of difference between two proportions was used to assess significant of difference between highly sensitive and resistant cases. P-values below and equal to 0.05 were considered significant statistically.

Results

Results showed that 14 of 435 samples were diagnosed as *Pantoea* spp. by API 20 E. Seven of the isolates were studied molecularly and diagnosed by PCR using 16S rRNA.

The findings are illustrated in Figure 1 showing that the colonies of *Pantoea* spp. on MacConkey agar were smooth, circles, convex, bright, moderate size and with pink color. Under microscope, they are Gram negative rod shape. *Pantoea* spp. were described biochemically by using API 20E tests. These assays identified these fourteen isolates under study as belonging to the *Pantoea* genus (Fig. 2). The results of the API 20 E test was summarized in Table 4.

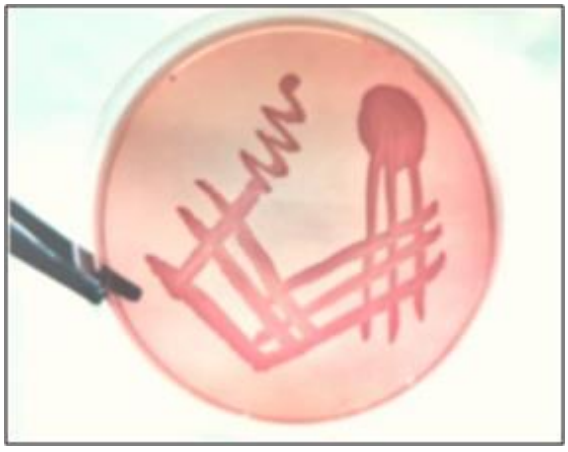

Fig. 1. *Pantoea* spp. growth on MacConkey agar

Fig. 2. API 20 E strip results for *Pantoea* spp.

Table 3
PCR optimal condition for genes under study

Name of genes	(Denaturation) – 1	(Denaturation) – 2	(Annealing)	(Extension) – 1	(Extension) – 2
16S rRNA	94 °C/ (5 min. 1 cycle)	94 °C/ (35 sec. 35 cycle)	57 °C/ (35 sec. 35 cycle)	72 °C/ (35 sec. 35 cycle)	94 °C/ (5 min. 1 cycle)
Esal	94 °C/ (5 min. 1 cycle)	94 °C/ (35 sec. 35 cycle)	59 °C/ (35 sec. 35 cycle)	72 °C/ (35 sec. 35 cycle)	94 °C/ (5 min. 1 cycle)
(*ompA*)	94 °C/ (5 min. 1 cycle)	94 °C/ (35 sec. 35 cycle)	55 °C/ (35 sec. 35 cycle)	72 °C/ (35 sec. 35 cycle)	94 °C/ (5 min. 1 cycle)

Table 4
Results of API 20E test for *Pantoea* spp.

Test No.	Test name	Result	Test No.	Test name	Result
1	ONPG	–	11	GEL	–
2	ADH	–	12	Glucose	+
3	LCD	–	13	MAN	+
4	ODC	–	14	INO	+
5	CIT	+	15	SOR	+
6	H_2S	–	16	RHA	+
7	Urease	–	17	SAC	+
8	TDA	–	18	MEL	+
9	IND	–	19	AMY	+
10	VP	+	20	ARA	+

The data shows that the highest percentage of *Pantoea* spp. was isolated from wound swabs, followed by urine samples and ear swabs: 5.00%, 1.88% and 1.33%, respectively (Table 5). Results also showed that the prevalence of *Pantoea* spp. infections in males more than females 9 (64%), 5 (36%), respectively (Fig. 3). Statistically, no significance was detected in terms of differences in *Pantoea* spp. infection according to the gender.

Table 5
The percentage of *Pantoea* spp. that isolated from clinical samples

Type of clinical specimen	Number of sample	Number of *Pantoea* isolates	Percentage
Urine	160	3	1.88
Pus (wounds)	200	10	5.00
Pus (ear)	75	1	1.33

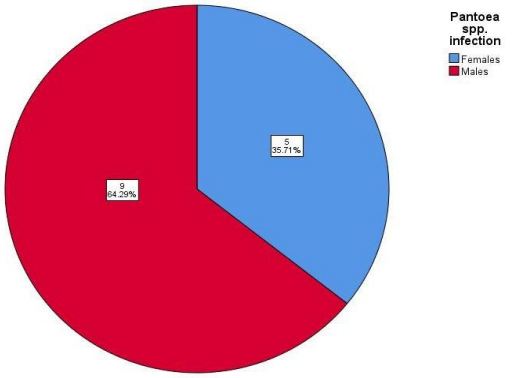

Fig. 3. Prevalence of *Pantoea* spp. infections in males and females

The results of antimicrobial susceptibility are summarized in Table 6 and Figure 4 shows the effectiveness of antibiotics against *Pantoea* spp., which explains that the meropenem was more effective against *Pantoea* spp. isolates 12/14 (85.7%) than other antibiotics, followed by cefotaxime 9/14 (64.3%), and less than fifty percent of isolates were susceptible to ciprofloxacin and tetracycline 6/14 (42.9%), while all isolates were resistant to tobramycin and clindamycin 14/14 (100%).

Table 6
Susceptibility test percentage of *Pantoea* spp. (number of isolates 14)

Antibiotics	Sensitive, No. (%)	Moderate sensitive, No. (%)	Resistant, No. (%)	P-value
Cefotaxime	9 (64.3)	–	5 (35.7)	0.2
Ciprofloxacin	6 (42.85)	2 (14.3)	6 (42.85)	0.3
Chloramphenicol	4 (28.6)	–	10 (71.4)	0.09
Gentamicin	5 (35.7)	4 (28.6)	5 (35.7)	0.9
Tetracycline	6 (42.85)	2 (14.3)	6 (42.85)	0.3
Amikacin	4 (28.6)	–	10 (71.4)	0.09
Meropenem	12 (85.7)	–	2 (14.3)	0.008
Trimethoprim/ Sulfamethoxazole	3 (21.4)	–	11 (78.6)	0.03
Tobramycin	–	–	14 (100)	–
Clindamycin	–	–	14 (100)	–
Cefalexin	2 (14.3)	–	12 (85.7)	0.008
Nalidixic acid	–	–	3(100)	–
Nitrofurantoin	3(100)	–	–	–

The findings demonstrate that the DNA extracted from seven isolates of bacteria under study is pure, as shown in Figure 5 and Table 7.

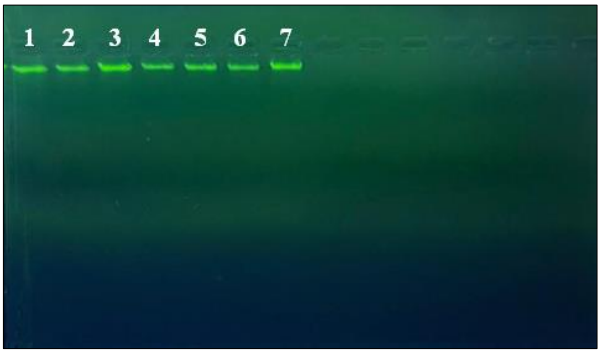

Fig. 5. Gel electrophoresis of genomic DNA extracted from *Pantoea* spp., agarose gel 1% at 5 vol/cm/30 min.

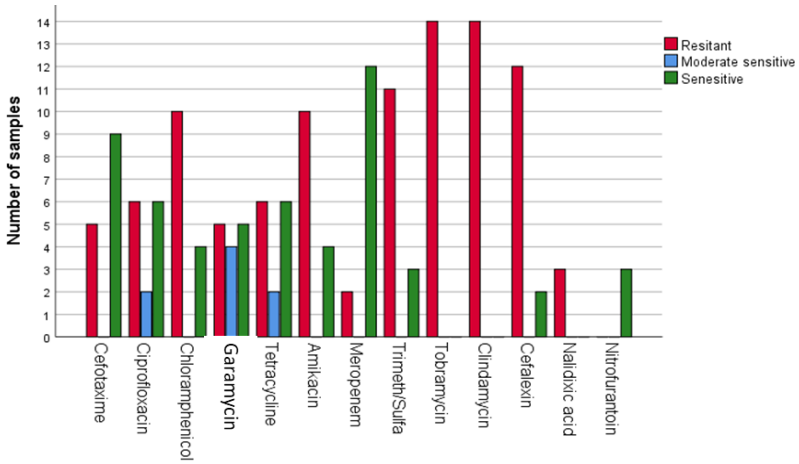

Fig. 4. The effectiveness of antibiotics against *Pantoea* spp.

Table 7
The purity and amount of DNA measured by nanodrop spectrophotometer

Strain No.	Conc. of nucleic acid, ng/mL	purity (260/280)
1	22.0	1.901
2	25.8	1.988
3	17.9	1.918
4	14.0	1.806
5	16.5	1.889
6	26.1	1.840
7	10.7	1.854

Seven isolates of *Pantoea* spp. underwent molecular detection by PCR assay, results explain that the isolates had 16S rRNA at a molecular weight of 445 bp. (Fig. 6), also isolates had *Esal* gene and *OmpA* gene at 301 bp, 202 bp, respectively, as shown in Figures 7 and 8, and Table 8.

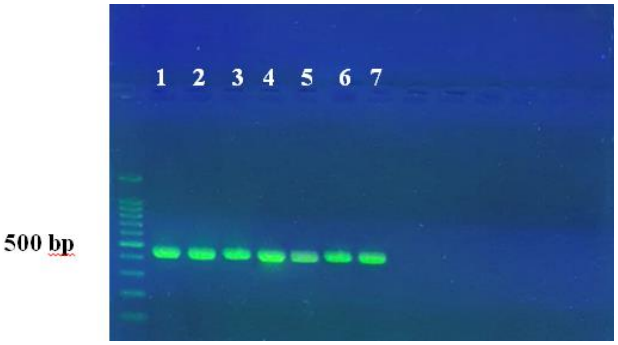

Fig. 6. 16S rRNA PCR product by electrophoresis, 445 bp.: band size at 5 volt/cm^2 on 1.5% agarose; (1xTBE) buffer for one hour and half, ladder DNA (100) bp.

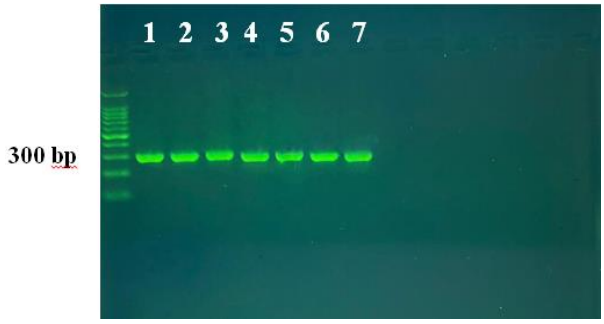

Fig. 7. *Esal* gene PCR product by electrophoresis, 301 bp. band size at 5 volt/cm^2 on 1.5% agarose; (1xTBE) buffer for one hour and half, ladder DNA 100 bp.

Fig. 8. *OmpA* gene PCR product by electrophoresis, 202 bp. band size at 5 volt/cm^2 on 1.5% agarose; (1xTBE) buffer for one hour and half, ladder DNA (100) (INTRON)

16S rRNA gene sequencing results for two isolates of *Pantoea* spp. under study explained that the match percentage of identities were 87% and 84%, respectively, as shown in Table 9. The results of *Esal* gene sequencing explained that the match percentage of identities was 67% and 68%, respectively, as shown in Tables 10. Table 11 shows the percentage of identities of *OmpA* gene sequencing of one *Pantoea* isolate was 88%.

Table 8
DNA extraction, PCR-16S rRNA, *Esal* and *OmpA* genes

Isolates No.	DNA extraction	PCR-16srRNA-455 bp.	PCR-*esal*-301 bp.	PCR-*ompA*-202 bp.
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+
5	+	+	+	+
6	+	+	+	+
7	+	+	+	+

Table 9
16S rRNA gene sequencing of *Pantoea* spp.

No.	Substitution type	Location	Nucleotide	Compare sequence ID	Source	Identities, %
1	Multiple mutations			ID: MH 883987.1	*Pantoea* spp. strain PS9 16S ribosomal RNA gene	87
2	Multiple mutations			ID: MH 883987.1	*Pantoea* spp. strain PS9 16S ribosomal RNA gene	84

Table 10
Esal gene sequencing of *Pantoea* spp.

No.	Substitution type	Location	Nucleotide	Compare sequence ID	Source	Identities, %
1	Multiple mutations			ID: OY 970450.1	*Pantoea* spp. Nvir isolate PANNVG genome assembly,	67
2	Multiple mutations			ID: OY 970450.1	*Pantoea* spp. Nvir isolate PANNVG genome assembly,	68

Table 11
OmpA gene sequencing of *Pantoea* spp.

Type of substitution	Location	Nucleotide	Compare sequence ID	Source	Identities, %
Transition/ Transversion	4077318	(C,G)s/T	ID: CP 117199.1	*Pantoea* spp. SS70 chromosome	88
Gap	4077318-920	(A,C)m			
Transition	4077320	a/G			
Transition	4077333	a/G			

Figure 9 explains the scheme of the sequence alignment for the phylogenetic tree analysis of closely related *Pantoea* spp. The numbers at the branches represent the percentage of compatibility. In our results, the numbers from 1–5 correspond to isolated *Pantoea* species. S1 = PQ303794.1 *Pantoea* spp. Iraqi 1, S3 = PQ303795.1 *Pantoea* spp. Iraqi 2.

Discussion

The genus *Pantoea* represents a group of bacteria (Gram-negative) in the family Erwiniaceae. *Pantoea* spp. are represented as environmental bacteria and regarded as plant pathogens, but also cause human infections and can be isolated from clinical samples. Many virulent bacteria also can be isolated from the natural environment (Hasan & Shakir, 2025). *Pantoea agglomerans* is the most widely described species in the literature as a saprophytic bacterium responsible for many human infections (Borrego et al., 2020; Koester et al., 2020; Cobo et al., 2022; Zahraa et al., 2023). In this work, the results of bacterial morphological demonstrates that *Pantoea* spp. on MacConkey media appeared as pink colonies due to their ability to ferment lactose sugar and produce alkaline in media (Okwundu & Mercer, 2019).

Pantoea spp. were identified biochemically by API 20E tests, which represent the first identification system to be used in laboratories of microbiology, combining a database and a strip of biochemical tests (Mahon & Lehman, 2019). The results of the current study showed that all fourteen isolates under study were found to fit the description of the *Pantoea* spp., they were positive to citrate and indole tests, while negative to urease, decarboxylate lysine, ornithine and Voges-Proskauer tests. By using the API 20E assay, bacterial isolates diagnosed at genus-level demonstrated that the API 20 technique supplies an exact and typical diagnosis for *Pantoea* spp. (Al-Kidsawey et al., 2020).

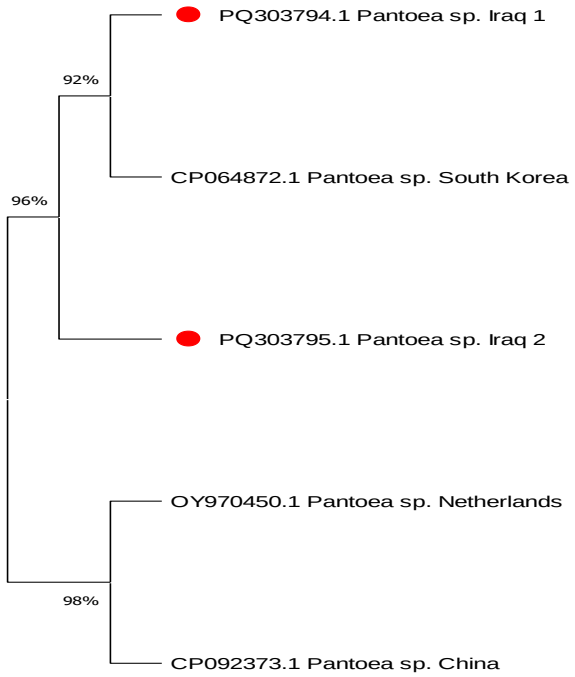

Fig. 9. Phylogenetic tree according to the sequences of the 16S rRNA gene for *Pantoea* spp.: red circles indicate isolates from this study, and other strains represent reference from NCBI database

Our study results revealed that the highest percentage of *Pantoea* spp. was isolated from wound swabs, results which are consistent with the findings of Jaber & Abdulmuhsen (2023). The current results also show that the percentage of *Pantoea* spp. isolated from urine samples was 1.88. In contrast to these findings, another investigation showed that the percentage of *Pantoea* spp. isolated from urine was (14%) (Fazza & Darweesh, 2020). Our results showed that the prevalence of *Pantoea* spp. infections was higher in males than in females. This may be due to the nature of their work, that males have being exposed to the environment in general and to plants in particular more than females. In contrast to these findings, another investigation reported that these bacteria caused urinary tract infection in females more than in males (Jaber & Abdulmuhsen, 2023). Also there are many types of infections that these bacteria can cause.

The results of antibiotics susceptibility tests found that meropenem significantly affected *Pantoea* spp. (P-value 0.008). These findings support those reported by Casale et al. (2023). Our results also show that the isolates of *Pantoea* species had high resistance aganst a broad range of antibiotics, including chloramphenicol, amikacin (71.4%) and trimethoprim / sulfamethoxazole (78.6%). These results are agreement with Fazza & Darweesh (2020), who found that *Pantoea* spp. are resistant to trimethoprim/sulfamethoxazole (72.4%). The antibiotic resistance results are also supported by those reported by Mardaneh & Pouresmaeil (2024). The reason of multi antibiotics resistance is that *Pantoea* spp. have many virulence genes such as *OmpA* and *Esal*. Therefore, researchers have focused on studying the problem of antibiotic resistance in many pathogenic bacteria and are trying to find solutions (Al-Bayati et al., 2025; Gopalraaj & Velayudhannair, 2025).

The outer membrane of bacteria forms as impermeable barrier for different antimicrobial proteins of body's immune system. There are many classes of outer membrane proteins in Gram-negative bacilli (Zhao et al., 2023). The main component of outer membrane proteins is an outer membrane protein A (*OmpA*), which has many functions which increase the pathogenicity of bacteria, and plays an important roles in supporting and anchoring of the outer membrane to the cell wall of bacteria, also it functions as an invasin and adhesin and plays a role in the formation of biofilm. The *Esal* gene is responsible for producing a typical (acyl-homoserine lactone) signal synthase. Gram-negative bacteria use (AHLs) as autoinducers. The mutation in this gene affects the pathogenicity of bacteria (Du et al., 2018; Vergalli et al., 2020).

The preparation of pure DNA has an A260/A280 ratio of equal to or greater than 1.8. But if the DNA is contaminated with RNA, that the ratio will be more than 2, while when it is contaminated with proteins the ratio will be less than 1.8 (Tamura et al., 2021). The results of our study showed clear bundles of extracted DNA from bacterial isolates, which indicates the purity of the DNA. The purity and concentration were determined, purity was between 1.06 and 1.988, and the nucleic acid concentration was between 10.7 and 26.1 ng/mL.

The 16S rRNA gene exists in most microorganisms. It is used as the universal for diagnosis and taxonomy of microorganisms. It is highly conserved between different species of bacteria. By using PCR assay for identify *Pantoea* spp. the results of study found that 16S rRNA exists in seven isolates at a molecular weight of 445 bp., which indicates the importance of 16S rRNA for diagnosis of the studied bacteria, and also the high sensitivity rates of PCR for isolating *Pantoea* spp.

Pantoea spp. have virulence genes *(Esal* and *OmpA*) responsible for pathogenesis, our findings indicated that the isolates under molecular study have these two genes (Table 8). The distribution of *Esal* and *OmpA* genes was 100% (7/7).

The results of (16S rRNA gene) sequence alignment showed multiple mutations of this gene, with 87% and 84% similarities of two isolates from the study with the *Pantoea* spp. strain PS9 (ID: OY970450.1) in Gen Bank, as shown in Table 9. Study revealed also multiple mutations in the *Esal* gene sequence, and the similarities of the two isolates were 67% and 68% with the *Pantoea* spp. strain Nvir PANNVG (ID: OY970450.1) in Gen Bank, as shown in Table 10. This difference in *Esal* gene sequence was registered in NCBI Submission No.: PQ303794 (National Library of Medicine). And through *OmpA* gene sequence alignment there were point mutations, results showed two genetic variations (as C/T Transition and G/T Transversion) of the *OmpA* gene at site 4077318, and the similarity of *Pantoea* spp. under study was (88%) with *Pantoea* spp. SS70 chromosome (ID: CP 117199.1) in Gen Bank, as shown in Table 11. Also analysis results explained the existence of Gap in location 4077318-920, and there were point mutations as A/G Transition in each of the sites 4077320 and 4077333 of the O*mpA* gene. This difference in *OmpA* gene sequence was registered in NCBI Submission No.: PQ303795 (National Library of Medicine).

According to the sequences of 16S rRNA gene, the phylogenic tree analysis of bacteria is used to find the closest relationship between bacterial species (Nakano et al., 2023). The tree was obtained by Maximum Composite Likehood, using BioNJ algorithms and Neighbor-Join. When comparing bacterial strains between different countries, the evolutionary tree in Figure 9 shows that the South Korean strain shares 92% identity with Iraq 1, showing moderate similarity, which may indicate a common evolutionary origin or similar environmental conditions. The Dutch and Chinese strains are very similar (98%), which probably indicates a very close relationship.

In future, more studies are needed to manifest the importance of *Esal and OmpA* genes in increasing the pathogenesis of *Pantoea* species, and their antibiotics resistance.

Conclusion

The current study investigated the distribution and prevalence of the *Pantoea* spp. in causing different infections of patients at Al-Sa-

lam, Al-Jumhori and Ibn Sina hospitals of Mosul. Fourteen strains were isolated and diagnosed by API 20 E and 16S rRNA gene sequences. This study demonstrates that the bacteria under investigation infect males more than females, and showed resistance to many antibiotics. The technique of PCR showed the existence of genes responsible of virulence (*Esal* and *OmpA*), which were important in causing bacterial infections. Genetic variation was examined and showed multiple mutation of (16S rRNA, esal) genes, and through *OmpA* gene sequence alignment there were point mutations.

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