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Research Article

Genetic study of *IntI1* and *IntI2* resistance genes and multidrug resistance of *Proteus mirabilis* isolated from clinical isolates from patients in Diyala province, Iraq

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Abstract
Proteus mirabilis has become a prevalent opportunistic pathogenic agent in clinical infections globally. The objective of this study was to analyze and discover some of genes responsible for antibiotic resistance in clinical isolates of *P. mirabilis* that exhibit resistance to multiple drugs, including *IntI1, IntI2* and to assess the relationship in the phylogenetic tree among these genes in Iraq, comparing to world-wide strains in National Center for Biotechnology Information (NCBI). A total of 250 samples from different sources were isolated in some Diyala province hospitals from August 2023 to January 2024 to be identified for the presence of *P. mirabilis* by biochemical and genetics test. The study demonstrated that 75 isolates were *P. mirabilis* 35 isolates (46.7%) from urine, (10.7%) from wounds, (13.3%) from burns and diabetic foot and 12 isolates (16%) from vaginal swabs and tested against 15 antibiotics for susceptibility. The prevalence of multi drug- resistance (MDR) in this study was high (97%) while only 2 isolates was tended to be extensively drug -resistance (XDR) (3%) no pan-drug –resistance(PDR) was detected. From 12 isolates of *p.mirabilis* 9 *IntI1* and 6 *IntI2* genes were detectable. homology between the sequenced sample of P. mirabilis reference target sequences. These genes were sequenced and phylogenetic relationships among these genes and global genes were documented in NCBI. The study suggests that some Iraqi isolates contain genetic variation compared to global strains and demonstrated the utility of *IntI1, IntI2* sequencing in investigating the genetic diversity of *P. mirabilis* strains and offering insights into the evolutionary history of these crucial bacterial pathogens.

Keywords: Antibiotics, extensively drug-resistant (XDR), GenBank, *IntI1, IntI2,* Mullti drug-resistant (MDR), *P. mirabilis*, virulence factors

INTRODUCTION

Proteus mirabilis is a motile, rod-shaped, non-capsulated, non-spore-forming, non-lactose fermenter member of gram-negative Enterobacteriaceae family, facultative anaerobe known for its urease production and the capability to ferment maltose and incapability to ferment lactose (Jamin *et al*., 2024). It is the most common species belonging to the genus Proteus and responsible for 80-90% of infections (Al-Kaim and Al-Dahmoshi, 2023). These bacteria have developed antibiotic-resistance strategies by forming new genes transferred through plasmids and other mobile genetic elements such as transposon and enteron (Evans *et al*., 2020). The genome of *Proteus mirabilis* has several genes that encode proteins responsible for creating antibiotic resistance, leading to the emergence of multi-drug-resistant and extensively drug-resistant species (Kanzari *et al.,* 2018). These genes, known as integrons (*IntI1*, *IntI2*), can be found on chromosomes or plasmids and can transfer antibiotic resistance horizontally among strains of bacteria. In the current study various genes were identified that contribute to antibiotic resistance in *Proteus mirabilis*, enabling it to resist multiple groups of antibiotics such as beta-lactam, quinolones, chloramphenicol, trimethoprim, aminoglycoside, and rifampicin. This multidrug resistance in *P. mirabilis* concerns the World Health Organization, as it classifies this pathogen as a medically significant nosocomial and community-acquired microorganism (Firmo *et al*., 2020). The present study aimed to identify *P. mirabilis* in various clinical samples, determine its viru-

lence factors and detect the existence of specific genes (IntI2,IntI1) containing a mobile genetic element in multidrug-resistant *P. mirabilis* found in clinical samples and investigate the relationship between these antibiotic-resistance genes in the phylogenetic tree.

MATERIALS AND METHODS

Isolating and identifying *Proteus mirabilis*

This cross-sectional study was performed in Baquba Teaching Hospital, Al-Batool Teaching Hospital, and AlKhalis hospital in Diyala province from August 2023 to January 2024. A total of 250 specimens were collected from urine samples, wounds, burns, diabetic foot, and vaginal swabs. Initially, samples were streaked on blood agar and MacConkey agar media to initiate primary isolation. The isolates were primarily identified as *P. mirabilis* based on their morphological, biochemical, and cultural characteristics, following the categorization described in Bergey's manual (MacFaddin, 2000). The conclusive identification was accomplished using a specific HiIMViC biochemical test kit. The verified *P. mirabilis* samples were 75 clinical isolates.

Ethical approval

The present research got the approval from the Medical Research Ethics Committee of Scientific Committee in the Department of Biology / College Sciences - University of Diyala (Ministry of Health or Hospital administration / (Iraq). All the needed information for patients or their parents was taken using local and simplified terms for a disease in their common language and invite them to be part of this research. The research is in accordance with the Ethical approval.

Antibiotic susceptibility test

The seventy five isolates were tested for antimicrobial susceptibility against fifteen antibiotics (Aztreonam 30µg/ml, Imipenem 10µg/ml, Cefoxitin 30µg/ml, Amikacin30µg/ml, Ceftazidime 30µg/ml, Ciprofloxacin 5µg/ml, Levofloxacin 5µg/ml, Ofloxacin 5µg/ml, Nalidixic acid 30µg/ml, Gentamicin 10µg/ml, Kanamycin 30µg/ml, Amoxicillin-clavulanic acid 20/10 µg/ml, Oxacillin 1µg/ml, Cefepime 30µg/ml, Azithromycin 15µg/ml and identified as either resistant or sensitive according to the standards set by the Clinical and Laboratory Standard Institute (2023) criteria (CLSI, 2023).

Detection of some virulence factors

Urease production

The isolates of *P. mirabilis* were cultured on Urea agar medium by stabbing and developed under controlled conditions (37ºC for 24-48 hrs.). The change of color from yellow to magenta was a sign of a positive result (Al-Mayahi, 2017).

Biofilm formation

The test for biofilm formation was conducted using the Microtiter plate method described by Ghellai *et al*. (2014). An ELISA reader was used to measure the concentration at 630 nm. The control well's OD value was subtracted from all the test OD values (ODc). Based on absorbance, the results were categorized into three groups: non-biofilm "OD" ≤ "OD", moderate "ODc < OD ≤ 2 x ODc", and strong "2 x ODc < OD". The biofilm formation test was carried out using the Microtiter plate method.

Detection of β-Lactamase production

A combination double-disk synergy (CDDST) test was used for screening of β-Lactamase by using piperacillin (PI) and piperacillin-tazobactam (TPZ) disk (Kalantar-Neyestanaki *et al*., 2017).

Detection of extended spectrum β-Lactamase

ESβL production in *P. mirabilis* was identified by the double disk synergy test (CDDST) by using Augmentin (20 µg amoxicillin and 10 µg of clavulanic acid) disk placed in the center of the plate and ceftazidime (30 µg), Aztreonam (30 µg), Cefotaxime (30 µg) and Piperacillin were positioned 3 cm apart center to center on the plates (Patel *et al*., 2017).

Molecular study

Molecular study was done to know the distribution of *IntI2, IntI1* among 12 *P. mirabilis* isolates.

Primer used in this study

Accurate primers were used for gene detection (*IntI2, IntI1*), as shown in Table 1 and PCR thermocycler program as shown in Table 2 .

Sequencing of resistant gene

The PCR results of resistance genes (*IntI1, IntI2*) were subjected to electrophoresis on a gel alongside 100 bp

Table 1. Primers used to detect the genes of *Proteus mirabilis*

Primer Name	Primer sequences	Annealing Temp. °C	Product size	Reference
IntI1-F	5`-CAGTGGACATAAGCCTGTTC-3	59	160 bp	(Abed *et al.,* 2022)
IntI1-R	5`-CCCGAGGCATAGACTGTA-3			
IntI2-F	5`-CACGCATATGCGACAAAAAGGT-3	55	788 bp	
IntI2-R	5`-GTAGCAAACGAGTGACGAAATG-3			

Table 2.PCR thermocycler program for *Proteus mirabilis* target gene

Steps	°C	m: s	Cycle
Initial denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	54	00:30	30
Extensions	72	01:00	30
Final extensions	72	07:00	1
Holds	10	10:00	1

m: minute; s: second

Table 3 . Distribution of *P. mirabilis* isolates across clinical sources

Type of samples	No. of isolates (%)	No. of total samples(%)	% of from total sample according to each type	P value & Chi-square
Urine	35 (46.7)	50 (20)	70	
Wounds	8 (10.7)	55 (22)	14	
Burns	10 (13.3)	55 (22)	18.2	
Diabetic foot	10 (13,3)	50 (20)	20	P value =0.000 Chi-sq = 47.43
Vaginal	12 (16)	40 (16)		
Total	75 (100)	250 (100)		

P value 0.05; Chi-sq =3.8

DNA ladder marker. Subsequently, the genes underwent nucleotide sequencing, and certain significant gene sequence were recorded in NCBI database. A particular and comprehensive tree was generated using the neighbor-joining technique. The observed variants were compared to their neighboring homologous reference sequences using the NCBI-BLASTn server.

Statistical analysis

The statistical analysis of this study was done using SPSS program. The results were assessed by One-Way Chi-square and $p<0.05$ value was considered significant.

RESULTS

Detection and characterization of *Proteus mirabilis*

Out of 250 clinical samples, 75 isolates were identified as *P. mirabilis* (30%) comprising of 35 distinct samples (46.7%) obtained from urine, 8 isolates (10.7%) obtained from wound, 10 isolates (13.3%) obtained from burns and diabetic foot and 12 isolates (16%) obtained from vaginal swabs (Table 3).

Antibiotic resistance pattern

A total of 75 *P. mirabilis* isolates analyzed to identify the presence of multidrug and extensive drug resistance against 15 antibiotics, namely Aztreonam, Imipenem, Cefoxitin, Amikacin, Ceftazidem, Ciprofloxacin, Levofloxacin, Ofloxacin, Nalidixic acid, Gentamicin, Kanamycin, Amoxicillin-clavulanic acid, Oxacillin, Cefepime, and Azithromycin, demonstrated the capacity of *P. mirabilis* to withstand drugs at varying levels, as shown in Table 4.

It was found that the majority of the isolates were multi-drug-resistant since they were resistant to three antibiotics or more. The prevalence of multi drug-resistance (MDR) *P. mirabilis* was high. Seventy-three (97%) of all isolates were MDR, while only 2 isolates tended to be XDR (3%), and no Pan Drug Resistance(PDR)was detected.

Virulence factors of *Proteus mirabilis*

Urease production

The present results showed that all of the isolates of *P. mirabilis* were phenotypically positive (100%).

Biofilm formation

The present study showed that all the isolates could form biofilm (100 %) in varying degrees Table 5.

β-lactamase and ESβ-lactamase production Enzymes

The results revealed that (37%) of *P. mirabilis* isolates the production β-lactamase fig. (1) and the results showed that 55 (73%) isolates of *P. mirabilis* producing ESβ-lactamase fig. (2).

DNA Extraction and gene detection of *Proteus mirabilis* isolates

The present results indicated that the extracts DNA concentration ranged from (30-70 ng/ µl). It was also observed that the purity of DNA extracts was satisfactory ranging from 1.55-1.98 according to the value rate of 260/280 nm. *P. mirabilis* isolates were discovered to have detectable *Intl1*in 9 (75%) of isolates. Single band was observed at a given molecular weight (160 bp). The results revealed that 6 (50%) of studied isolates have *Intl2* gene. Single band was observed at a given molecular weight (788bp) (Fig. 3 and 4). One bacterial isolate, *P. mirabilis* number (2) isolated from Diabetic Foot infection was identified and submitted to the Na-

Table 4. Antibiotics discs for *Proteus mirabilis* used in this study.

Antibiotics	R	%	I	%	S	%
Imipenem IMI	13	17	7	9.3	55	73
Ceftazidime CAZ	45	60	23	30	7	10
Cefixime CX	2	3	13	17	60	80
Kanamycin K	47	63	11	37	17	22.7
Augmentin AUG	20	27	25	33	30	40
Ofloxacin OFX	8	10	2	3	65	87
Levofloxacin LEV	2	3	2	3	71	94.7
Amikacin AK	75	100	0	0	0	0
Nalidixic acid NA	25	33	2	3	48	64
Genetamicin CN	38	50	13	17	24	32
Norfloxacin NX	0	0	0	0	75	100
Ciprofloxacin CIP	2	3	13	17	60	80
Oxacillin OX	75	100	0	0	0	0
Azetronam AT	35	47	7	23	33	44
Amoxicillin AX	73	97	0	0	2	3

R: Resistant; I: Intermediate; S:Susceptible;

Table 5. Biofilm production test in *Proteus mirabilis*

Biofilm	Strong	Moderate	Weak	Total
No.	28	47	0	75
Percentage	37 %	63 %	0 %	100%

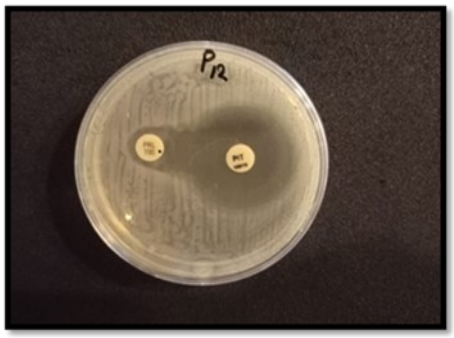


Fig. 1. *β-lactamase produce by the combination double disk synergy test (CDDST)*

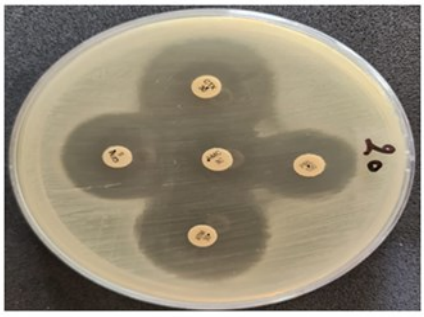


Fig. 2. *ESβ-lactamase produce by the double disk synergy test (DDST)*

tional Center for Biotechnology Information (NCBI) with the accession number as shown in Table 6.

Sequence in Multi Drug Resistance *Proteus mirabilis* genes

Four samples (assigned B1 to B4) were amplified from the *IntI* locus. The sequencing reactions indicated the exact identity after performing NCBI blastn for these PCR amplicons. The NCBI BLASTn engine showed 100% homology between the sequenced sample of B1 – B4 and *P. mirabilis* reference target sequences (GenBank acc. KP66515.1), and the recovered PCR fragments exact positions and other information were identified. The overall length of the specific regions of interest was calculated using the NCBI server, and the precise start and end positions of these regions were verified based on the most closely related bacterial target, as shown in Fig.5b. Four samples, designated as C1 to C4, were amplified from the IntI2 gene. The PCR amplicons were subjected to NCBI blastn analysis, which revealed the precise identification of the sequences. The NCBI BLASTn engine revealed a homology of around 99% between the sequenced sample of C1 – C4 and the reference target sequences of *P.mirabilis* (GenBank acc. CP138492.1). The exact positions and other information of the retrieved PCR fragments were recognized. The overall length of the specific loci was determined on the NCBI server, and the precise positions of the beginning and end of the targeted loci were verified according to the (Fig.5 c).

After setting the PCR amplicons' sequences of *IntI1, IntI2* within the genomic sequences of *P. mirabilis*, the information of its sequences was pointed out, and the overall length of the amplified amplicons was obtained as well (Table 7).

Concerning *IntI1* sequences, the alignment of the

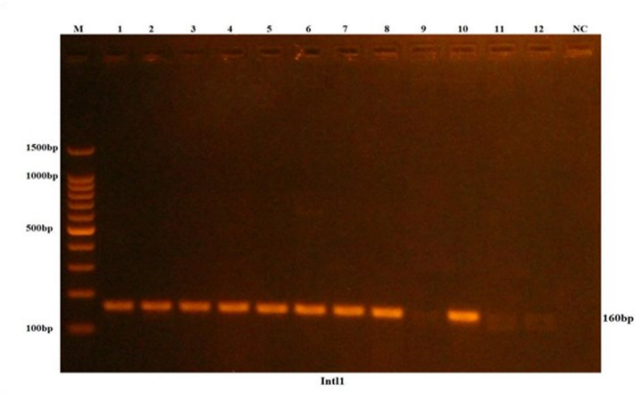


Fig. 3.*PCR amplification of the IntI1gene from Proteus. mirabilis, with the amplicon size 160bp. DNA amplification products were separated by electrophoresis in (2%) agarose gel. Electrophoresis was performed at 70 volt for 1.5 hour. Symbol "M" refers to ladder marker*

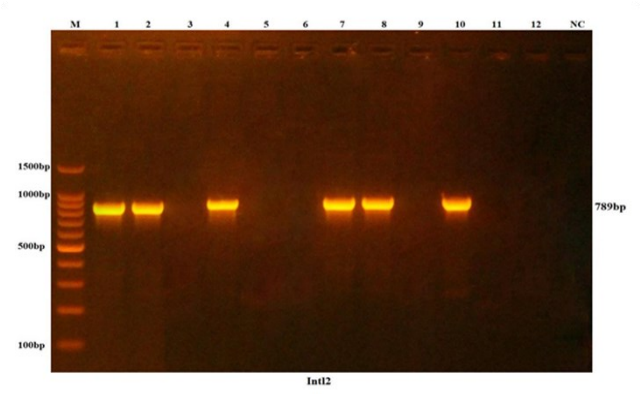


Fig. 4. *PCR amplification of the IntI2gene from Proteus mirabilis, with the amplicon size 788bp. DNA amplification products were separated by electrophoresis in (2%) agarose gel. Eelectrophoresis was performed at 70 volt for 1.5 hour. The symbol "M" refers to ladder marker*

Table 6. Standard strain of *Proteus mirabilis* isolate No.2 isolated from Diabetic foot infection

Strain's Name	Key characteristic	Source	GenBank Accession Number
P. mirabilis	*IntI1*	This study	LC810417.1
P. mirabilis	*IntI2*	This study	LC810418.1

Table 7. Details of the identified variants in the investigated sequences that are partially covered *IntI1* (branch B*), IntI2* (branch C), sequences within *Proteus mirabilis*, respectively. The identified amino acid variants are named according to the international nomenclature system. The letter "p." refers to the "protein". Arginine), which can affect protein function

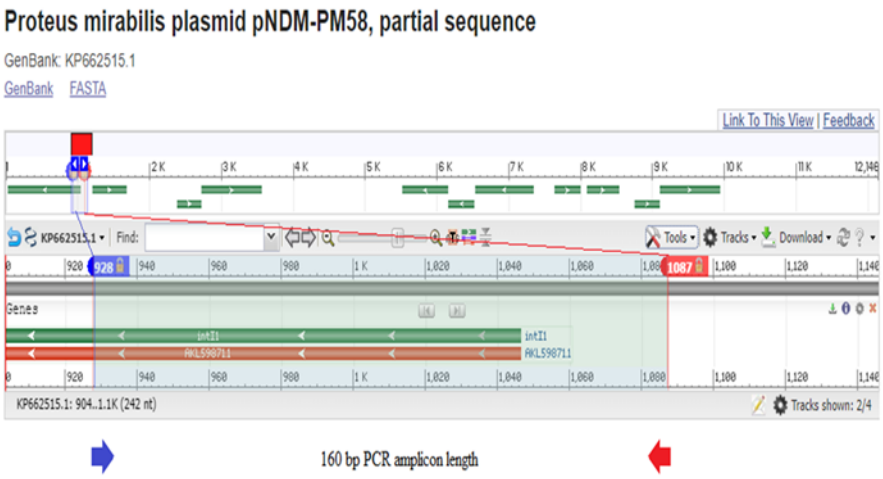
No.	Sample	Position in PCR amplicon	variant	Single Nucleotide position (SNP)	Position in protein	consequences
B)	*IntI1*					
	no variation detected	-	-	-	-	-
C)	*IntI2*					
	C4	199	T>A	199T>A	77	Silent SNP (p. Ala77=)

investigated B1 – B4 samples showed the lack of any nucleic acid variations compared to the most similar referring reference nucleic acid sequences (Fig. 5b). In *IntI2* sequences, the alignment of the investigated C1 – C4 samples showed the presence only one nucleic acid variation comparing to the most similar referring reference nucleic acid sequences. The sequencing results confirmed the existence of no nucleic acid variants in the B1 – B4 samples compared with the reference sequences of P. mirabilis (GenBank acc. no. KP662515.1) (Fig. 5b). The sequencing reactions also confirmed the identification of only one nucleic acid variants (199T>A) identified within the C1 – C4 samples compared with the same reference sequences (GenBank acc. no. CP138492.1) (Fig. 5c).

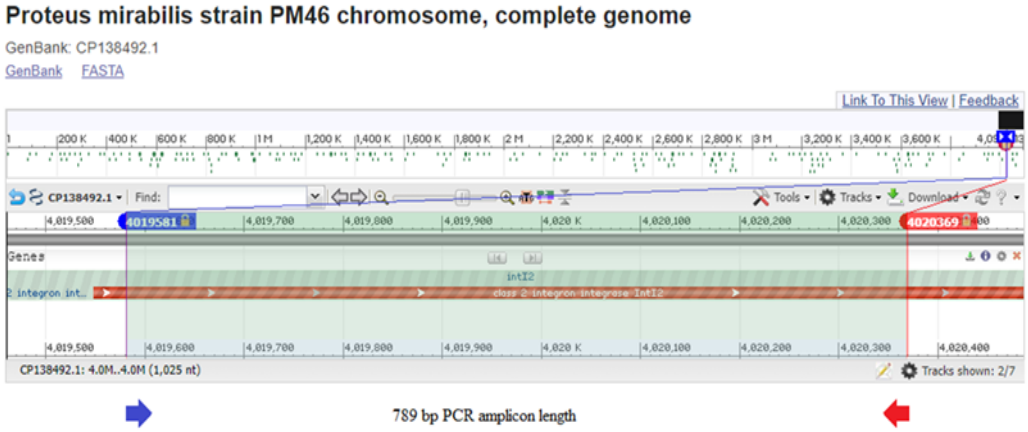
No variations were identified in the *IntI1* amplicon since it was found that the amplified products of the *IntI1* gene have covered 39 amino acid residues within the bacterial integrase (Fig. 5b). Results from the direct nucleic acid translation of the identified variants of 199T>A showed a silent impact on the integron integrase *IntI2*, namely p. Ala77= (Fig. 5c). Presents data from a study on the genetic variations found in the genes of *Proteus mirabilis*. The position in the protein column is applicable to the nucleotide change. For instance, "77" refers to the 77th amino acid within the protein. The consequences column describes the impact of the variant on the organism or the genes function. "Silent SNP" means that despite the nucleotide change table (7), the amino acid remains the same (p. Ala77= means that at position 77, Alanine is unchanged). "Missense SNP" indicates a change in the amino acid sequence (113W>R means Tryptophan at position 113 is replaced Table 7.

Comprehensive phylogenetic tree of MDR of *P. mirabilis* :

In this study nucleic acid sequences generated in the amplifying PCR products the *IntI1, IntI2* amplicons of *P. mirabilis*. Phylogenetic trees are graphical representations of the evolutionary relationships among bacterial sequences based on their genetic information. In this case, the genetic diversity of these bacterial organisms can be analyzed by sequencing specific regions of the *IntI1, IntI2* sequences (Fig. 6 and 7).



IntI1 (branch B)



IntI2 (branch C)

Fig. 5. *Eact position of the investigated samples that are amplified in the study to partially cover IntI1 (branch B), IntI2 (branch C), sequences within Proteus mirabilis*

DISCUSSION

The study focused primarily on some resistant genes and pathogenicity factors of *P. mirabilis* isolates recovered from hospitals in different regions in Diyala and selected based on their susceptibility profiles and some virulence factors. The present study for biofilm formation agrees with Mirzeai *et al.,* (2019) who revealed that all isolates were biofilm producers. Biofilm formation enables bacteria to be protected from a range of stressors, such as immunological reactions and antimicrobial treatments. Bacteria that can produce biofilms are often more resistant to antibiotics. These biofilms can be formed by individual germs or by several microorganisms forming micro-communities. A crucial determinant of pathogenicity is the capacity of a microorganism to produce biofilm, which offers a protective niche for survival and resistance against antibiotics (Dincer *et al*., 2020). The present results for urease production agree with the results of Bunyan and Albakery, (2021) , who found that (100%) of *P. mirabilis* isolates showed strong urease production.This enzyme catalyzes the formation of kidney and bladder stones or to encrust or obstruct indwelling urinary (Yuan *et al.,* 2021). In the present study, 97% of the isolates were classified as MDR since they were resistant to at least three classes of antibiotics, and all of them belonged to isolates that were collected from inpatients. The significant prevalence of MDR and XDR strains among the studied isolates and their wide variety can be related to the horizontal transfer of resistance determinants among bacterial isolates in hospitals. The results agree with Al-Kaim and Al-Dahmoshi (2023), who recorded that 96% of their isolates were MDR. A dissimilar observation by (Ramatla *et al*., 2024) revealed that (30.7%) of the isolates were classified as MDR.

MDR may be mediated by resistance agents located on chromosomes or mutations in a resident gene. However, it may also expand by attaining resistance genes through horizontal transfer (AL-Fatlawi *et al*., 2023). In the present study, the susceptibility pattern of *ESBL*-producing isolates showed that these isolates were resistant not only β- lactams but also to other antibiotics, including fluoroquinolones, aminoglycosides and amoxicillin-clavulanic acid. The current study found a clear correlation between the occurrence of multidrug-resistant (MDR) bacteria and the production of extended-spectrum beta-lactamases (ESBLs). The MDR of *P.*

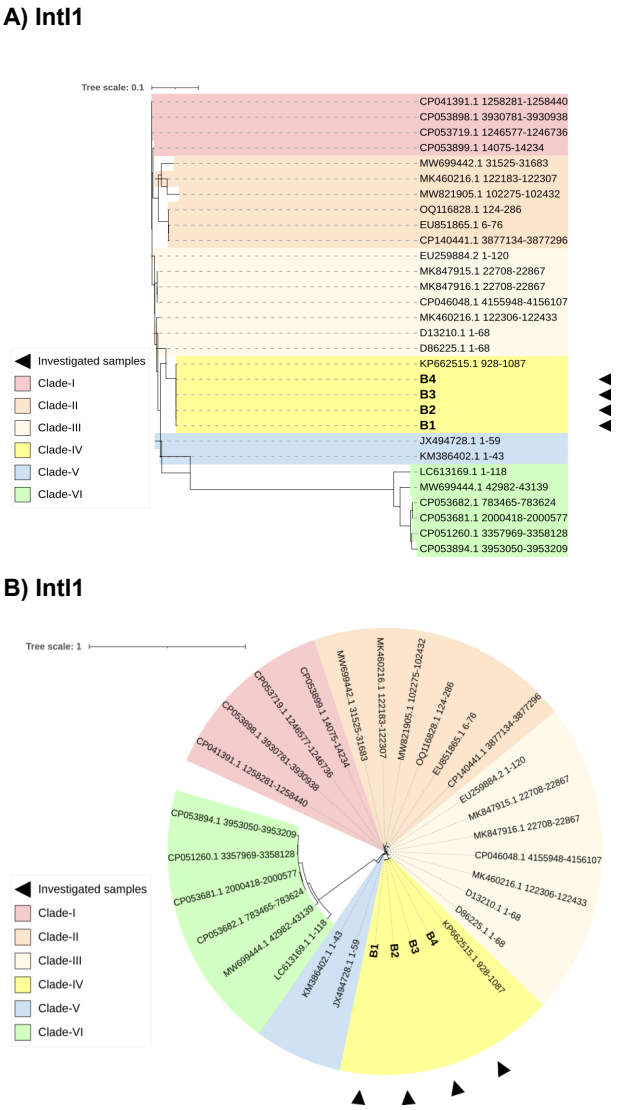


Fig. 6. *Comprehensive cladogram phylogenetic tree of the IntI1 fragments for four samples of P. mirabilis. The tree is presented in rectangular (branch A) and circular (branch B) cladograms. The triangles colored in black refers to the investigated bacterial sequences. Every specified number corresponds to the GenBank accession number for each reference species. The top part numbers of the tree indicate the degree of scale range among the comprehensive tree-categorized organisms*

Fig. 7. *Comprehensive cladogram phylogenetic tree of the IntI2 fragments for four samples of P. mirabilis. The tree is presented in rectangular (branch A) and circular (branch B) cladograms. The triangles colored in black refer to the investigated bacterial sequences. Every specified number corresponds to the GenBank accession number for each reference species. The top part numbers of the tree indicate the degree of scale range among the comprehensive tree-categorized organisms*

mirabilis strains were more commonly found in ESBL-positive isolates than ESBL-negative isolates. Furthermore, the majority of MDR isolates were also found to produce ESBLs. Studies have demonstrated a clear correlation between the resistance to antibiotics and the level of biofilm formation. This correlation may be attributed to the transfer of resistance genes through genetic elements within biofilms (Kot *et al*., 2021). Multidrug resistance of twelve *P. mirabilis* Isolates exhibit varying levels of resistance genes (*IntI1, IntI2*). These findings disagreed with other studies reported before by (AL-Fatlawi *et al*., 2023) in Kufa Hospital that showed (16.65%) of their studied isolates were harboring *IntI1* gene and these findings agreed with other studies reported earlier, such as Mirzaei *et al*. (2021) who showed that *IntI2* gene was found in (25%) of their studied isolates. The presence of these genes in *P. mirabilis* isolates confers resistance to various antibiotic groups, including β-lactam, aminoglycoside, and quinolones.

In addition to well-known bacterial mutation strategies, antibiotic genes are increasingly being captured in in-

tegron-borne cassettes, which provide an efficient mechanism for capturing and exchanging different resistance genes. Integrons are not self-transferable elements, however, they are frequently found on transposons or plasmids, enabling the successful transmission of genes. More than 100 gene cassettes bearing resistance to various classes of antibiotics have been reported (Chen *et al*., 2017). Class 1 integrons are the most clinically present, most closely associated with antibiotics, and have been most intensively studied (Sun *et al*., 2020). Class 2 integrons are often underappreciated and less studied because of the presence of a stop codon (TAA) on the integrase gene, making it non-functional to integrate, recombine, and cleave the drug resistance cassette (Lu *et al*., 2022). Sequencing analysis of their variable region revealed that they mainly carried distinctive cassettes encoding aminoglycosides and trimethoprim resistance determinants. Previous reports have also indicated that most integrons-carrying genes for aminoglycosides and trimethoprim resistance (Fursova *et al*., 2015). Variable insights into the diversity and relatedness of strains are obtained by comparing the genetic sequences obtained from *P. mirabilis* isolates from various sources.

The phylogenetic analysis allows for classifying *P. mirabilis* isolates into several distinct clades, which can provide information about the spread and transmission of specific strains. Using the currently generated tree, the closely related strains that may have emerged from a common ancestor are resolved. This can aid in obtaining a comprehensive understanding of the genetic diversity and population structure of *P. mirabilis*. Concerning the *IntI1* sequences, the complete tree had thirty aligned nucleic acid sequences. Interestingly, the only species of incorporated bacterial sequences was found to be attributed to *P. mirabilis*. This data also refers to the high specificity of this genetic fragment in identifying this type of bacterial sequence without confusion with the other related bacterial organisms. Within the produced cladogram, the included samples were grouped into six phylogenetic clades within *P. mirabilis.* The investigated B1 – B4 samples were incorporated with the clade-IV in the vicinity of one bacterial strain that is isolated from China (GenBank KP662515.1). This clade originated from the adjacent clade (clade-III) since clade-III is positioned in the vicinity of the roots of the tree. The same thing is also applicable for the clade -I. However, it seems that the *IntI1* gene is characterized by its high level of genetic variations among the *P. mirabilis* strains. This is due to the variable phylogenetic positioning found among the incorporated clades. Nevertheless, all the investigated B1 – B4 samples were incorporated within one clade within this tree (Fig.6).

Concerning the *IntI2 s*equences, the overall number of aligned nucleic acid sequences in this comprehensive tree was_twenty-six. Interestingly, the only species of incorporated bacterial sequences was attributed to *P. mirabilis*. As in the case of the previous loci, this data refers to the high specificity of this genetic fragment in the identification of this type of bacterial sequence without confusion with the other related bacterial organisms. Within the produced cladogram, the studied samples were grouped into four phylogenetic clades within *P. mirabilis*. The investigated C1 – C4 samples were incorporated with the clade-1 in the vicinity of various bacterial strains that are mainly isolated from China (GenBank CP053614.1, CP053719.1, CP045538.1, CP046048.1, CP053684.1, CP051260.1, CP053681.1, MK670976.1, and MK670980.1). However, one strain isolated from Canada (GenBank CP048404.1) as also identified in the same clade. Accordingly, the Asian - European regions are the expected origins from which our investigated samples originated. Clade I was positioned near Clade II, which was made of three samples of *P. mirabilis*. Due to its close positioning toward the roots of the tree, the clade-I was found to be attributed to the ancestors from which the clade II, III, and I Voriginated (Fig.7).

The trees present observation confirmed the sequencing reactions accuracy by revealing the exact neighbor-joining-based structure of the investigated sequences .The utilization of *IntI1, IntI2*. Sequences in this study has provided further indication for precisely these bacterial organisms .The sequences considered in this study have provided additional proof for precisely identifying these bacterial organisms.

The present study demonstrated the utility of *IntI1 and IntI2* sequencing in investigating the genetic diversity *of p.mirabilis* strains and offered insights into the evolutionary history of these crucial bacterial pathogens. Overall, emphasizing the importance of understanding the genetic variations present in bacterial samples and their impact on the evolutionary relationships between different groups of *P. mirabilis* is highly significant for assessing the biological diversity of each *P. mirabilis* isolate.

Conclusion

The results revealed that the biofilm formation level was linked with antibiotic susceptibility. Also, the study found that the high rate of MDR and the emergence of XDR among the tested isolates their enormous diversity could be explained by the horizontal transfer of resistance determinants among seventy-five bacterial isolates from different sources, urine, wound, burn diabetic foot and vaginal swabs of patients in hospitals. The detection of resistance and identification *IntI1, IntI2* genes in clinical *P.mirabilis* isolates in patients of Diyala teaching hospitals were recorded for the first time in Diyala province.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Abed, G. E., Salim, H. A. & Fadhil, H. W. (2022). Study of Some Resistance Genes in BMC veterinary research, 16, 1-10.Clinical *Proteus mirabilis.* https://doi/10.22092 ari.2022.358489.2230
2. AL-Fatlawi, M. R., Zwain, O. M. & Alquraishi, Z. H. (2023). Role of Integrons 1 and 2 in Proteus spp. and Evaluation of Some Antibiotic's Resistance Genes. *Egyptian Academic Journal of Biological Sciences. C, Physiology and Molecular Biology*, 15(2), 367-377. https://doiI: 10.21608/ EAJBSC.2023.319284.
3. Al-Kaim, H. W., & Al-Dahmoshi, H. O. (2023). Prevalence of AcrAB-TolC efflux pump among *Proteus mirabilis* isolated from cystitis patients in Hilla City, Iraq. *Medical Journal of Babylon*, *20*(3), 503-510.
4. Al-Mayahi, F. S. (2017). Phenotypic and Molecular detection of Virulence factors in Proteus mirabilis isolated from different clinical sources. Bas J Vet Res, 16(1), 369-88
5. Bunyan, I. A., & Albakery, S. A. (2021). Molecular Detection of Some Virulence Factors of Uropathogenic Proteus mirabilis. International journal of Information Technology, 40, 536-545. https://doi.org/10.36295/ASRO.2021.24616
6. Chen, C. M., Lai, C. H., Wu, H. J., & Wu, L. T. (2017). Genetic characteristic of class 1 integrons in proteus mirabilis isolates from urine samples. *BioMedicine*, 7(2). https://doi: 10.1051/bmdcn/2017070202
7. CLSI (2023). Performance Standards for Antimicrobial Susceptibility Testing, in CLSI supplement M100, 33th ed (Wayne, PA, USA: Clinical and Laboratory Standards Institute.
8. Dincer, S. Uslu, F.M. and Delik, A. (2020). Antibiotic Resistance in Biofilm. In Bacterial biofilms. IntechOpen.
9. Evans, D. R., Griffith, M. P. Sundermann, A. J. Shutt, K. A. Saul, M. I. Mustapha, M. M. & Van Tyne, D. (2020). Systematic detection of horizontal gene transfer across genera among multidrug-resistant bacteria in a single hospital. Elife, 9, e53886. . https://doi.org/10.7554/ eLife.53886.
10. Firmo, E. F., Beltrão, E. M. B. da Silva, F. R. F. Alves, L. C. Brayner, F. A. Veras, D. L. & Lopes, A. C. S. (2020). Association of *blaNDM*-1 with *blaKPC*-2 and aminoglycoside-modifying enzyme genes among Klebsiella pneumoniae, *Proteus mirabilis* and *Serratia marcescens* clinical isolates in Brazil. *Journal of Global Antimicrobial Resistance*, 21, 255-261. https://doi.org/10.1016/ j.jgar.2019.08.026.
11. Fursova, N. K., Astashkin, E. I., Knyazeva, A. I., Kartsev, N. N., Leonova, E. S., Ershova, O. N., ... & Dyatlov, I. A. (2015). The spread of bla OXA-48 and bla OXA-244 carbapenemase genes among *Klebsiella pneumoniae*, *Proteus mirabilis* and *Enterobacter spp.* isolated in Moscow, Russia. *Annals of clinical Microbiology and Antimicrobials*, 14, 1-9.https://doi .1186/s12941-015-0108-y.
12. Ghellai, L., Hassaine, H. Klouche, N. Khadir, A. Aissaoui, N. Nas, F. & Zingg, W. (2014). Detection of biofilm formation of a collection of fifty strains of *aureus* isolated in Algeria at the University Hospital of Tlemcen. *Journal of bacteriology research*, *6*(1), 1-6.https:// doi: 10. *Staphylococcus* 5897/JBR2013.0122.
13. Jamin , K.J., Al-Dahmosi, H.O.& Jabuk, S.I.A. (2024). Molecular study of blaCTX-M profile among uropathogenic proteus mirabilis isolates. *Medical Journal of Babylon*, 21(1),137-143. https://doi: 10.4103/ MJBL.MJBL_494_23.
14. Kalantar-Neyestanaki, D., Koshesh, M. Hashemizadeh, Z. Mansouri, S. Bahador, A. & Savari, M. (2017). The β-Lactamase Disk Test: A Modified Method to Detect Extended-Spectrum-β-Lactamases in Multidrug-Resistant *Escherichia coli* Isolates. Archives of Clinical Infectious Diseases, 12(1).https:// doi.org/10.5812/archcid.39070.
15. Kanzari, L., Ferjani, S. Saidani, M. Hamzaoui, Z. Jendoubi, A. Harbaoui, S. & Slim, A. (2018). First report of extensively-drug-resistant Proteus mirabilis isolate carrying plasmid-mediated *blaNDM*-1 in a Tunisian intensive care unit. *International journal of antimicrobial agents*, 52 (6), 906-909. https://doi.org/10.1016/j.ijantimicag.2018.06.009.
16. Kot, B., Grużewska, A. Szweda, P. Wicha, J. & Parulska, U. (2021). Antibiotic resistance of uropathogens isolated from patients hospitalized in district hospital in central Poland in 2020. Antibiotics, 10(4), 447. https:// doi.org/10.3390/antibiotics10040447.
17. MacFaddin, J. F. (2000). Biochemical tests for identification of medical bacteria. 3rd ed. Lippincott Williams and Wilkins, Philadelphia, PA, 363-367.
18. Mirzaei A., Habibi M. Bouzari S. Asadi Karam MR. (2019). Characterization of Antibiotic-Susceptibility Patterns, Virulence Factor Profiles and Clonal Relatedness in *Proteus mirabilis* Isolates from Patients with Urinary Tract Infection in Iran. Infection and Drug Resistance, 12: 3967–3979. https://doi.org/10.2147/IDR.S230303.
19. Mirzaei, A., Nasr Esfahani, B., Raz, A., Ghanadian, M., & Moghim, S. (2021). From the urinary catheter to the prevalence of three classes of integrons, β□lactamase genes, and differences in antimicrobial susceptibility of Proteus mirabilis and clonal relatedness with Rep□PCR. *BioMed research international*, *2021*(1), 9952769. https:// doi.org/10.1155/2021/9952769.
20. Lu, W., Qiu, Q. Chen, K. Zhao, R. Li, Q. & Wu, Q. (2022). Distribution and molecular characterization of functional class 2 integrons in clinical Proteus mirabilis isolates. *Infection and Drug Resistance*, 465-474. https:// doi.org/10.2147/IDR.S347119.
21. Patel, J.B., Weinstein, M. Eliopoulos, G. Jenkins, S. Lewis, J. Limbago, B. Mathers, A.J. and Mazzulli, T. (2017). M100 Performance standards for antimicrobial susceptibility testing. United State: Clinical and Laboratory Standards Institute (CLSI), p.240.
22. Ramatla, T., Ramaili, T. Lekota, K. Mileng, K. Ndou, R. Mphuthi, M. & Thekisoe, O. (2024). Antibiotic resistance and virulence profiles of Proteus mirabilis isolated from broiler chickens at abattoir in South Africa. *Veterinary Medicine and Science*, 10(2), e1371. https://doi: 10.1002/ vms3.1371.
23. Sun, Y., Wen, S. Zhao, L. Xia, Q. Pan, Y. Liu, H. & Wang, H. (2020). Association among biofilm formation, virulence gene expression, and antibiotic resistance in Proteus mirabilis isolates from diarrhetic animals in Northeast China. https://doi.org/10.1186/s12917-020-02372-w
24. Yuan, F., Huang, Z. Yang, T. Wang, G. Li, P. Yang, B. & Li, J. (2021). Pathogenesis of *Proteus mirabilis* in catheter -associated urinary tract infections. *Urologia Internationalis*, 105(5-6), 354-361. https://doi: 10.1159/000514097 .