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Research Article

Molecular characterization of antibiotic resistance and virulence genes on plasmids of *Proteus mirabilis* isolated from urine samples of Hospitals in Mosul City, Iraq

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Abstract
Antibiotic resistance genes when found on plasmids can be passed on to other strains causing spread of antibiotic resistance across bacteria. The present work aimed to identify virulence and resistance genes on *Proteus mirabilis* plasmids. A total of 37 *P. mirabilis* strains were isolated from 420 urine samples from patients attending different hospitals in Mosul City, Iraq, from December 2022 to April 2023 and identified using biochemical and molecular methods. Their resistance towards 18 antibiotics was tested and their plasmid DNA analysis showed that 21 of 37 *Proteus* strains contained plasmids. Four groups of primers were used for PCR experiments. The first group included primers used to identify six genetic regions, namely (CITM, DHAM, ACCM, EBCM, FOXM, MOXM1). Results showed that 85.71% of isolates carried FOXM on their plasmids and 14.28% carried MOXM and 4.76% carried CITM. However, DHAH, ACCM, and EBCM were not detected on plasmids. The second group included *zapA*, *ireA* (siderophore receptor), *hpmA* (hemolysin) and *mrpA*, (fimbriae) genes. zapA was detected in 80.95% of *P. mirabilis* plasmids, followed by *ireA* at a rate of 76.19%, *hpmA* at 14.28%, and *mrpA* at 4.76%. The third group included CTXG1, CTXG2, CTXG9, CTXG8 and CTXG25. The results showed that CTX9 was the highest gene detected, 76.19%, followed by CTXG1 71.42%, while CTXG2, CTXG8, and CTXG25 were only detected on the chromosome. Finally, the pathogenic genes *PmlJ1* and qnrD were found on *P. mirabilis* plasmids at 52.38% and 47.61%, respectively. The present results showed that plasmids are increasingly spread among clinical local isolates of *P. mirabilis*, and serious precautions are required.

Keywords: Antibiotic resistance genes, *Proteus mirabilis*, Plasmids, Virulence genes

INTRODUCTION

Bacterial infections have become a major healthcare challenge owing to the rise and dissemination of multi-drug-resistant bacteria. Over the last few decades, *Proteus* infections have received special attention due to the emergence of species resistant to various antimicrobial agents, particularly β-lactams. According to the World Health Organization (Jun Kwon *et al*., 2022; Vasconcelos *et al*., 2018), antimicrobial drug abuse is a universal challenge.

After *Escherichia coli* and *Klebsiella pneumoniae, Proteus* isolates are the third most frequent cause of urinary tract infections (UTIs). It mainly accounts for severe UTIs or UTIs continuously catheterized individuals. *Proteus* species are widespread soil inhabitants and part of the normal flora of human and animal intestinal regions and have been shown to cause opportunistic infections in various anatomical areas (Talebi *et al.,* 2023; Wang *et al.,* 2023).

This bacterium is on the list of medically significant nosocomial agents because of its virulence factors, including antibiotic resistance genes, fimbria, flagella, hemolysins, urease, proteases, amino acid deaminase, lipopolysaccharides (LPS), and capsular polysaccharides (Shanmugasundarasamy *et al*., 2022). Antibiotic resistance in bacterial infections is growing, increasing the likelihood of therapeutic failure and death (Hutinel *et al*., 2022).

Conjugative Plasmids are crucial to the physiology of bacteria, and they are the primary agents for horizontal gene transfer (HGT) in clinical settings, which facilitate

the direct exchange of resistance genes that aid bacteria to quickly adjust to therapeutic surroundings (Hua *et al*., 2020). Recent research has revealed that more plasmids than previously thought may be able to mobilize and undergo HGT (Ramsay and Firth, 2017). Mobile genetic elements particularly insertion sequences play a role in the rearangment of plasmids (Hua *et al*., 2020). Although all Extended Spectrum Beta Lactamases (ESBLs) function by cleaving the amide bond of the beta-lactam ring, the genes encoding these enzymes are diverse and divided into several groups (Biondo, 2023).

Until 2000, TEM- and SHV-type ESBLs were the predominant ESBL families. Today, the most commonly encountered ESBL types are phylogenetically distinct from the first β-lactamases that appeared in the early 1980s (Hays *et al*., 2022). CTX-M-type enzymes are the most commonly encountered ESBL types present in several members of the order Enterobacteriales (Yasmeen *et al*., 2023). Isolated isolates harboring CTX -M had great resistance to cefotaxime and low susceptibility to ceftazidime (Mushtaq, 2022). Other forms of ESBLs include OXAs, AmpCs, and carbapenemases. Oxas and AmpC are β-lactamase enzymes encoded by chromosomal and plasmid genes. They are resistant to β-lactamase inhibitors. The most prevalent mechanism of bacterial resistance is medication efflux from cells via membrane transporters. These transporters are proteins from the ATP-binding cassette (ABC) gene superfamily. Overexpression of ABC transporters is a major determinant of multidrug resistance (Zheng and Lupoli, 2023; Wu *et al*., 2023).

UTIs are mostly caused by Gram-negative bacteria, which pose a growing concern to public health due to their potential to acquire genes from transferable plasmids that code for extended-spectrum -lactamases (ESBLs) (Bedenić and Meštrović, 2021). These enzymes can hydrolyze third-generation cephalosporins and monobactams, but not carbapenems. Furthermore, ESBLs constitute a public health hazard since they are encoded on plasmids that typically carry other resistance genes against several types of antibiotics, including aminoglycosides, sulfonamides, and quinolones (Mancuso *et al*., 2021). Plasmids promote the transfer of genetic material, including antimicrobial resistance genes, between bacterial species and genera. Because of the presence of virulence factors on these mobile genetic components, bacterial populations may become more virulent (Abdulrahman and Omar, 2012). *P. mirabilis* coordinates an increase in the synthesis of many virulence factors, such as the hemolysin. Like other Enterobacterales, clinical strains of *P. mirabilis* have developed increased resistance to antimicrobial drugs over the past few decades (Filipiak *et al*., 2020 ; He *et al*., 2019).

The ability of this organism to produce a range of extracellular enzymes, including urease, which causes kidney and bladder stones to develop, may account for some of its medical significance. Stones around the bacterium prevent antibiotics from having their intended therapeutic effects. Additionally, hemolysin is toxic to the cells that line the urinary tract (Abbas *et al*., 2015). Adhesins, toxins, invasins, protein secretion systems, iron absorption systems, and other bacterial virulence factors may be encoded on the chromosomal DNA of *P. mirabilis*, bacteriophage DNA, plasmids, or transposons (Shelenkov *et al*., 2020 ; Carattoli, 2013).

The (KPC) enzyme hydrolyzes most β-lactam antibiotics, including carbapenems. Numerous KPC variants have been reported, and KPC- producing bacteria have been found worldwide. These bacteria often belong to the order Enterobacterales (Hua *et al*., 2020a). Although the prevalence of carbapenemase in *P. mirabilis* is relatively low, it tends to rise globally over time. KPC synthesis is frequently linked to other resistance mechanisms, and strains containing these enzymes typically have strong resistance to fluoroquinolones and aminoglycosides (Girlich *et al*., 2020; Castanheira *et al*., 2010). *Proteus* has long been recognized to be responsive to β-lactam antibiotics. Extended-spectrum β-lactamase has led to increased resistance (Musa *et al*., 2019). Plasmid-mediated CTX-M enzymes are the most frequent ESBLs, and studies have revealed that the genes encoding for CTX-M β-lactamases were more prevalent among tested bacterial strains compared with the genes encoding SHV-type or TEM-type β-lactamase (Ojdana *et al*., 2014; Qin *et al*., 2015).

Due to the importance of plasmids in *P. mirabilis* isolates causing UTI, the present study aimed to detect the number of plasmids found in the isolates and identify the genes commonly located on such plasmids. For this purpose, 17 pairs of primers were used to amplify several virulence and antibiotic-resistant genes predicted to be carried on *P. mirabilis* plasmids.

MATERIALS AND METHODS

Sample collection and bacterial identification

Four hundred and fifty urine samples were collected from patients of different ages suffering from urinary tract infections (UTI) who visited Ibn Sina Teaching Hospital, Al Salam Teaching Hospital, Al Jumhuri Teaching Hospital, and Mosul General Hospital in Mosul city, Iraq, from December 2022 to April 2023. Sediments from urine were streaked on blood and MacConkey agar plates. The plates were incubated aerobically at 37°C for 24 h. Isolates that produced smooth, non-lactose fermenting colonies on MacConkey agar and swarmed on blood agar were selected for further identification (Hayat *et al*., 2023). Standard biochemical tests, including catalase and oxidase, were used for species identification (Vandepitte *et al*.,2003).

Molecular identification of *Proteus mirabilis* isolates by Polymerase chain reaction

Genomic DNA was extracted from pure cultures of *P. mirabilis* isolates using the genomic DNA isolation kit supplied by Geneaid (Taiwan). Steps were followed as recommended by the manufacturer. The concentration and purity of genomic DNA were measured, and DNA was stored at 20°C until further use.

For molecular identification, PCR was carried out in a 20 µL reaction volume using GoTaq G2 Green Master Mix provided by Promega (USA). The full region of the 16S rRNA gene was amplified employing the universal primers 27F (5' AGAGTTTGATCMTGGCTCAG 3') and 1522R (5' AAGGAGGTGATCCARCCGCA 3'), as suggested by Abdulrazzaq and Faisal (2022). The primer concentration was 1 µM each, and 100 ng of DNA template was added, adhering to the manufacturer's recommendations. The PCR program for the 16S rRNA gene involved an initial denaturation at 95°C for 3 min, followed by 30 cycles of amplification comprising denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min. After the final extension step at 72°C, PCR products were resolved on a 1% agarose gel and dyed with Midori Green Advance DNA stain. A 100-bp DNA marker from New England Biolabs, UK, served as the reference for molecular weight (Khaleel *et al*., 2023a).

The PCR products targeting the 16S rRNA gene were purified and forwarded to Psomagene sequencing company (USA) for sequencing. The obtained sequences were compared for similarity with published genes in GenBank using the BLAST tool at NCBI. For control purposes in plasmid experiments, the same primers were used as a control to detect possible contamination of plasmids with chromosomal DNA.

Antimicrobial susceptibility test

The Kirby Bauer disk diffusion method for antibiotic susceptibility was performed on all isolates under study, and the results were interpreted as per the CLSI 2022 recommendations (Lewis and James, 2022). Eighteen antibiotic disks were purchased from (Bioanalyse/ Turkey) and used to test *P. mirabilis* isolates: amoxicillin-clavulanate (20/10 µg/ disk), amoxicillin (10µg/ disk), ampicillin (25µg/ disk), Piperacillin (100/10 µg/ disk), cephalexin (30 µg/ disk), Cefotaxime (10 µg/ disk), Norfloxacin (30 µg/ disk), Imipenem (10 µg/ disk), meropenem (10 µg/ disk), gentamicin (10 µg/ disk), amikacin (10 µg/ disk), ciprofloxacin (10 µg/ disk), trimethoprim (10 µg/ disk) azithromycin (15 µg/ disk), chloramphenicol (10 µg/ disk), tetracycline (10µg/disk), nalidixic acid (30 µg/disk), and streptomycin (25 µg/ disk). An organism demonstrating resistance to at least one antibiotic from three or more categories of antimicrobial agents is labeled as multidrug resistant (MDR). Isolates sensitive to only one or two categories of antimicrobials fall under the classification of extensive drug-resistant (XDR) isolates, according to Magiorakos *et al*. (2012).

Molecular detection of virulence and antibiotic resistance genes in *P. mirabilis*

Genomic DNA was isolated from all *P. mirabilis* strains under study using a kit supplied by Geneaid (Taiwan). The 17 sets of primers listed in Table 1 for virulence and antibiotic resistance determinants in *P. mirabilis* were used to detect the corresponding genes in the genomic DNA of *P. mirabilis*. On the other hand, plasmid DNA was purified from multidrug-resistant *P. mirabilis* using the Large Plasmid DNA Extraction Kit supplied by Geneaid company (Taiwan) following the manufacturer's instructions. Plasmids were separated by electrophoresis on 2% agarose gel at 100 V for 1 h, visualized with a UV transilluminator, and plasmid bands were excised from agarose and purified using the gel extraction kit supplied by Geneaid (Taiwan) following their instructions. To detect the location of the antibiotic resistance genes and virulence-related genes, whether on the plasmid or on the bacterial chromosome, PCR was conducted on the purified plasmid DNA using the primers listed in Table 1 and an additional PCR reaction was performed using the universal 16S rRNA primers as a control.

RESULTS AND DISCUSSION

Identification of *P. mirabilis*

Biochemical and molecular identification based on 16S rRNA showed that 37/420 (8.8%) of the urine samples analyzed were positive for *P. mirabilis*. The morphological characteristics of *P. mirabilis* colonies on blood agar were distinctive due to their ability to swarm on the agar surface, whereas colonies were pale to colorless on MacConkey agar media and had the very weak swarming ability. All isolates were Gram-negative bacilli when examined microscopically (Mirzaei *et al*., 2019; Gupta *et al*., 2002). Molecular diagnosis using 16S rRNA primers produced the expected bands (Fig. 1), and when sequenced and blasted to the NCBI database, they were identified as *P. mirabilis*

Antibiotic susceptibility

The results obtained from the disk diffusion method for antibiotic susceptibility testing of *P. mirabilis* against 18 antibiotics were confirmed based on the diameter of the inhibition zone surrounding the disks and compared with the standard tables mentioned in (CLSI 2022). The results showed a clear difference in the resistance of the isolates under study to the antibiotics used (Fig. 2). Local isolates of *P. mirabilis* isolated from urine showed a high resistance to amoxicillin at 100%, which agrees with similar studies on *P. mirabilis* including Al-Taie *et al*. (2013) and two other studies by Wang *et al.* (2014)

Table 1. DNA sequences of primers used in this study

Genes	Sequences 5'-3'		Products size(bp)	PCR program	Source of primer
ireA	F	ACTACGATAACGAGCGCCAG	681		
	R	GCCCTAACTGGGGGAATACG		Initial denaturation : 95 °C for 5/ min	
hpmA	F	GTTGAGGGGCGTTATCAAGAGTC	709	Denaturation: 94 °C (5/ min)	(Sanches *et al*., 2019)
	R	GATACTGTTTTGCCCTTTTGTGC		Annealing : 58 °C for ireA ,zapA / 52° C for hpmA, mrpA (1/min)	
zapA	F	TATCGTCTCCTTCGCCTCCA	332	Extension72 °C (1/min)	
	R	TGGCGCAAATACGACTACCA		Final extension:72 °C for 7 min	
mrpA	F	GAGCCATTCAATTAGGAATAATCCA	648		
	R	AGCTCTGTACTTCCTTGTATACAGA			
MOXM	F	GCTGCTCAAGGAGCACAGGAT	520		
	R	CACATTGACATAGGTGTGGTG			
CITM	F	TGGCCAGAACTGACAGGCAAA	462		
	R	TTTCTCCTGAACGTGGCTGGC			
DHAM	F	AACTTTCACAGGTGTGCTGGGT	405	Initial denaturation :94 °C for 3min	
	R	CCGTACGCATACTGGCTTTGC		Denaturation: 94 °C (0:30 / min)	(Pérez-Pérez and Hanson, 2002)
ACCM	F	AACAGCCTCAGCAGCCGGTTA	346	Annealing : 58 °C (0:40/ min)	
	R	TTCGCCGCAATCATCCCTAGC		Extension72 °C (1/min)	
EBCM	F	TCGGTAAAGCCGATGTTGCGG	302	Final extension:72 °C for 7 min	
	R	CTTCCACTGCGGCTGCCAGTT			
FOXM	F	AACATGGGGTATCAGGGAGATG	190		
	R	CAAAGCGCGTAACCGGATTGG			
CTXG1	F	AAAAATCACTGCGCCAGTTC	415		
	R	AGCTTATTCATCGCCACGTT			
CTXG2	F	5-CGACGCTACCCCTGCTATT-3	552	Initial denaturation :94 °C for 5/ min	
	R	CCAGCGTCAGATTTTTCAGG		Denaturation: 94 °C (0:30/ min)	(Woodford *et al*., 2006)
CTXG9	F	CAAAGAGAGTGCAACGGATG	205	Annealing : 52 °C (0:40/min)	
	R	ATTGGAAAGCGTTCATCACC		Extension72 °C (1/min)	
				Final extension:72 °C for 6/ min	
CTXG-8	F	TCGCGTTAAGCGGATGATGC	327		
CTXG8-25	R	AACCCACGATGTGGGTAGC			
CTXG -25	F	GCACGATGACATTCGGG	666		
CTXG8-25	R	AACCCACGATGTGGGTAGC			
qnrD	F	CGAGATCAATTTACGGGGAATA	565	Initial denaturation :94 °C for 3min	(Cavaco *et al*., 2009)
	R	AACAAGCTGAAGCGCCTG		Denaturation: 94 °C (0:30 min)	
				Annealing : 50 °C (0:40/min)	
				Extension72 °C (1/min)	
				Final extension:72 °C for 7/ min	
PmlJ1	F	ACACCTACAACAAGGCTATC	400	Initial denaturation :94 °C for 3min	(Bie *et al*., 2018)
LJR1	R	AGTTCTAAAGGTTCGTAGTCG		Denaturation: 94 ° C (0:30 min)	
				Annealing : 49 ° C (0:40/min)	
				Extension72 °C (1/min)	
				Final extension:72 °C for 7/ min	
16S rRNA	27 F	AGAGTTTGATCMTGGCTCAG	1495	Initial denaturation: 95 °C for 3 min Denaturation: 95 °C for (0:30 min) Annealing at 55 °C (0:30 min) Extension 72 °C for 1 min.	(Abdulrazzaq and Faisal, 2022)
	1522R	AAGGAGGTGATCCARCCGCA		Final extension: 72 °C for 7/ min	

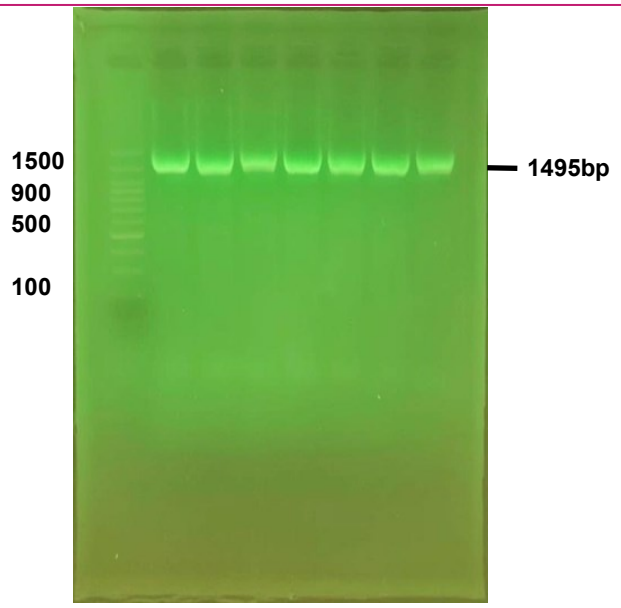

Fig.1.*16S rRNA Amplicons of selected isolates of Proteus mirabilis*

and Zixuan *et al.* (2022) whom found relatively high amoxicillin resistance percentages of 83.6% and 98%, respectively. Large numbers of the gene have always been altered in bacterial isolates that produce beta-lactamases (Oliver and On, 1999). The results also revealed a percentage of trimethoprim-sulfamethoxazole (97%), which is close to the results obtained by (Zixuan *et al*., 2022), who found that the resistance percentage among *P. mirabilis* was (98%). Other studies by Jun kwon *et al*. (2022) found that the resistance rate was (72%) among *P. mirabilis* isolates. Bacteria resist antibiotics by producing enzymes that modify or destroy antibiotics, making them ineffective. Bacteria can also modify the target of the antibiotic within their cells, making them less susceptible to its effects. In addition, bacteria may acquire mutations that alter their cellular functions, making them resistant to antibiotics. Understanding these mechanisms is crucial in developing strategies to combat antibiotic resistance (Girlich *et al*., 2020). The present study also detected high resistance to ampicillin (94.59%) and tetracycline (94.59%). The percentages are close to what was observed by Shabeeb *et al*. (2018), who demonstrated that (97.37%) of *P. mirabilis* isolates were resistant to ampicillin and 91.89% were resistant to piperacillin; this was similar to the study of Mo *et al*. (2022). Their results also showed that cefotaxime (a third-generation antibiotic) had a resistance rate of (86.48%), while cephalexin was 89.18%. Quinolone antibiotics showed a strong and effective effect on *P. mirabilis* isolates, as the resistance rate of bacterial isolates to nalidixic acid was 89.18%. In addition, the current study showed that the resistance of *P. mirabilis* to chloramphenicol was 83.78% and to azithromycin was 67.56%. Most of the resistance of bacteria to macrolide antibiotics occurs through inhibition of protein synthesis by affecting the large subunit of the 50S ribosome (Grossman, 2016; Butler *et al*., 2010).

The current study showed that the resistance of bacterial isolates to amoxicillin-clavulanic acid was 64.86%. This was similar to the results reached by John *et al*. (2022), where it was found that the resistance of *P. mirabilis* to this antibiotic was (63%). The current results showed that the percentage of isolates resistant to streptomycin was 67.56%, whereas bacterial isolates showed resistance to gentamicin at a rate of 62.16%. These antibiotics are considered a group of aminoglycosides commonly used in medical clinics to treat life-threatening infections caused by Gram-negative bacteria, and are the most common aminoglycosides used to treat UTI patients in many countries. One of the causes of resistance of *E. coli* to antibiotics belonging to the aminoglycosides group is the possession of efflux systems and a change in membrane permeability as well as the presence of aminoglycoside-modifying enzymes,

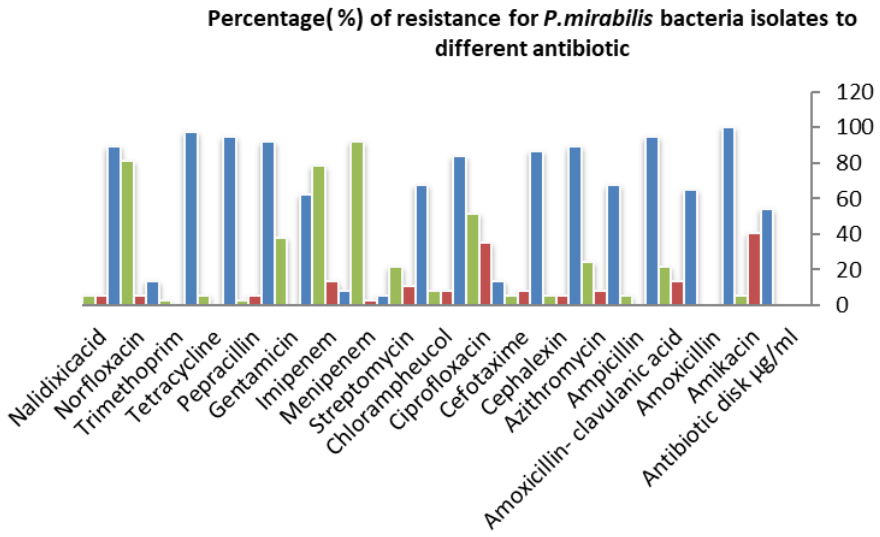

Fig 2. *Resistance levels of Proteus mirabilis isolates to different antibiotics*

N-acetyl transferase and phosphotransferase (Zaman *et al.,* 2017). This is almost inconsistent with the results obtained by Jun kwon *et al.* (2022), who found that the proportion of gentamicin was 22% among *P. mirabilis* isolates. The results also showed that the percentage of amikacin was 54.05%, which does not agree with the results obtained by John *et al*. (2022), who found that the resistance rate for amikacin was (16%) among *P. mirabilis* isolates.

Resistance of *P. mirabilis* to ciprofloxacin in our study was 13.51%, which is higher than that observed by Al-Marjani (2000), who found that the resistance toward ciprofloxacin in *P. mirabilis* was (4.7%), and Al-Tamimi and Jabbar (2021), who found it to be 8%. Interestingly, the percentage of resistance toward norfloxacin was identical to that of ciprofloxacin (13.51%), which determines that the same genes are responsible for the resistance of both antibiotics. Resistance of imipenem and meropenim were 8.10% and 5.40%, respectively. Tahreer *et al*. (2019) detected low resistance to these antibiotics towards *Proteus* spp., who recorded the resistance rate of imipenem and meropenem (0%). In general, the increase in bacterial resistance to most antibiotics may be the widespread and indiscriminate use of antibiotics and the lack of health awareness (Eisner *et al.,* 2006; Khaleel *et al.,* 2023b).

Molecular studies

In an attempt to search for virulence and antibiotic resistance genes on *P. mirabilis* plasmids, we detected plasmids from 21/37 (56.7%) *P. mirabilis* isolates. Primers for various antibiotic resistance and virulence genes that enable and contribute to the development of infection, particularly those involved in UTI invasion in humans, were used to search for corresponding genes in *P. mirabilis.* Our results showed that *all P. mirabilis* strains containing plasmids contained the selected genes at a percentage of 100% for all genes.

The primers used in this study were divided into four groups. The first group included the primers used to identify six genetic regions, namely CITM, DHAM, ACCM, EBCM, FOXM, MOXM. Results shown in Table 2 indicate that MOXM was detected in 14% of the bacterial isolate plasmids, whereas FOXM appeared in 85.7% of the plasmids. With respect to the primer CTIM, a clear band appeared from the plasmids of 4.7% of the isolates. The appearance of virulence genes in samples of bacteria isolated from urine indicates the virulence of the isolates and their resistance to most antibiotics. This confirms that *Proteus* have an important role in causing infections in the urinary tract, according to what was mentioned previously, which agrees with what was stated previously (Aryal *et al*., 2020; Pérez-Pérez and Hanson, 2002). They found that the genes encoding beta-lactamase enzymes were widespread among *Proteus* isolates, but their prevalence rate differed. This could be because genes carried on conjugative plasmids spread faster through conjugation, and genes differ from each other in their rate of selection through transformation. However, the genes DHAM, ACCM, and EBCM were not detected on *P. mirabilis* plasmids, indicating that they were chromosomally located. AmpC β-lactamases are cephalosporinases of clinical significance encoded on the chromosomes of numerous Enterobacteriaceae and a limited number of other organisms. They play a role in conferring resistance to cephlothin, cefazolin, cefoxitin, most penicillins, and combinations of β-lactamase inhibitors with β-lactams, as outlined by Hayat (2023).

The second group included *zapA*, *ireA* (siderophore receptor), *hpmA* (hemolysin) and *mrpA*, (fimbriae) genes. zapA was detected in 80.95% of *P. mirabilis* plasmids, followed by ireA at a rate of 76.19%, *hpmA* gene at 14.28% and *mrpA* gene at 4.76%. The presence of these genes on *P. mirabilis* plasmids indicates that the isolates use plasmid genes for antibiotic resistance and virulence. Antibiotic resistance via plasmids may cause higher levels of resistance in the isolates harboring them. Several studies have detected these genes on *P. mirabilis* plasmids (Sanches *et al.,*2019; Cestari *et al.,* 2013; Swihart and Welch, 1990). Li *et al*. (2012) noted that antibiotic resistance in *K. pneumoniae* isolated was widely spread. This is because the genes carried on conjugative plasmids spread faster through the conjugation process.

The third group included CTXG1, CTXG2, CTXG9, CTXG8, CTXG25,. The results shown in Fig. 4 show the appearance of the genetic regions of the five genes on *P. mirabilis* plasmids. CTX9 was the highest gene detected on plasmid at a percentage (76.19%) followed by CTXG1 (71.42%). This agrees with what was stated by Woodford *et al*. (2006). The other genes, CTXG2, CTXG8, and CTXG25, were detected in the genome but not on plasmids. CTX-M extended-spectrum β-lactamases (ESBLs) are becoming more widespread globally, particularly among *Escherichia coli*, *Klebsiella* spp., and *Proteus* spp. This prevalence extends to the United Kingdom, where more than half of microbiology laboratories have reported encountering ESBL producers (Woodford *et al*., 2004; Munday *et al*., 2004). More than 40 CTX-M β-lactamases have been described and are divided into five phylogenetic groups, with different groups prevalent in different countries (Bonnet, 2004).

Associated *PmIJ1* and qnrD genes that are linked to *P. mirabilis* pathogenesis were found on *P. mirabilis* plasmids at a rate of 52.38% and 47.61%, respectively, as shown in Table 2. *P. mirabilis* strains can produce many virulence factors that play an important role in human infection. Previous research has detected the qnrD gene in plasmids of *P. mirabilis* (Cavaco *et al*.,

Table 2. Sequences of primers used in the present study

Target	Sequence (5' - 3')	Isolates containing plasmids	Detection on plasmid (%)
CTIM	F 5-TGGCCAGAACTGACAGGCAAA-3 R 5-TTTCTCCTGAACGTGGCTGGC-3	No	1/21 (4.76)
DHAM	F 5-AACTTTCACAGGTGTGCTGGGT-3 R 5-CCGTACGCATACTGGCTTTGC-3	No	0/21 (0)
ACCM	F 5-AACAGCCTCAGCAGCCGGTTA-3 R 5-TTCGCCGCAATCATCCCTAGC-3	No	0/21 (0)
EBCM	F 5-TCGGTAAAGCCGATGTTGCGG-3 R 5-CTTCCACTGCGGCTGCCAGTT-3	No	0/21 (0)
FOXM	F5-AACATGGGGTATCAGGGAGATG-3 R5-CAAAGCGCGTAACCGGATTGG-3	2,4,6,10,12,13,15,17,20,23,26,29,30 ,32 ,34,35,36,37	18/21(85.71)
MOXM	F 5-GCTGCTCAAGGAGCACAGGAT-3 R 5-CACATTGACATAGGTGTGGTG-3	35,36,37	3/21(14.28)
qnrD	F 5-CGAGATCAATTTACGGGGAATA-3 R 5-AACAAGCTGAAGCGCCTG-3	6,13,15,17,20 ,29,32,35,36,37	10/21 (47.61)
zapA	F 5-TATCGTCTCCTTCGCCTCCA-3 R 5-TGGCGCAAATACGACTACCA-3	4,6,10,12,13,15,17,20,23,26,29,30, 32 ,34,35,36,37	17/21 (80.95)
ireA	F 5-ACTACGATAACGAGCGCCAG-3 R 5-GCCCTAACTGGGGGAATACG-3	4,6,10,12,13,15,17,20,23,26,29,30, ,34,35, 36,37	16/21 (76.19)
hpmA	F 5-GTTGAGGGGCGTTATCAAGAGTC-3 R 5-GATACTGTTTTGCCCTTTTGTGC-3	6,36 29،	3/21 (14.28)
mrpA	F 5-TGGCGCAAATACGACTACCA-3 R5-GAGCCATTCAATTAGGAATAATCCA-3	6	1/21 (4.76)
CTXG1	F 5-AAAAATCACTGCGCCAGTTC-3 R 5-AGCTTATTCATCGCCACGTT-3	2,4,6,12,13,16,20,23,26,29,30,32,34,36,37	15/21 (71.42)
CTXG2	F 5-CGACGCTACCCCTGCTATT-3 R 5-CCAGCGTCAGATTTTTCAGG-3	NO	0/21 (0)
CTXG9	F 5-CAAAGAGAGTGCAACGGATG-3 R 5-ATTGGAAAGCGTTCATCACC-3	2,4,6,12,16,17,20,23,26,29,30,32,34,35,36,37	16/21 (76.19)
CTXG8	F 5-TCGCGTTAAGCGGATGATGC-3 R 5-GCACGATGACATTCGGG-3	NO	0/21 (0)
CTXG25	F 5-TCGCGTTAAGCGGATGATGC-3 R 5-GCACGATGACATTCGGG-3	NO	0/21 (0)
PmlJ1 LJR1	F5-ACACCTACAACAAGGCTATC-3 R5-ACACCTACAACAAGGCTATC-3	2,4,6,10,12,13,15,17,20 ,26,32	11(52.38)

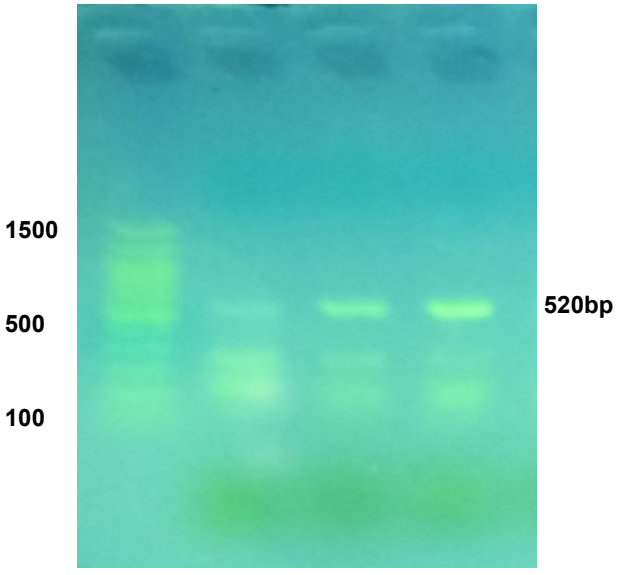

Fig. 3. *Multiplex PCR assay for MOXM gene (520 bp) and CTIM gene (462bp) detected on P. mirabilis plasmids*

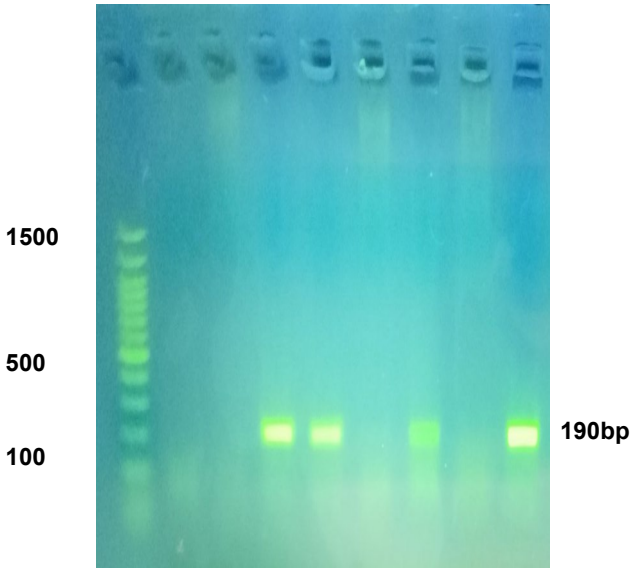

Fig. 4. *Amplicon of FOXM gene (190 bp) detected on P. mirabilis plasmids*

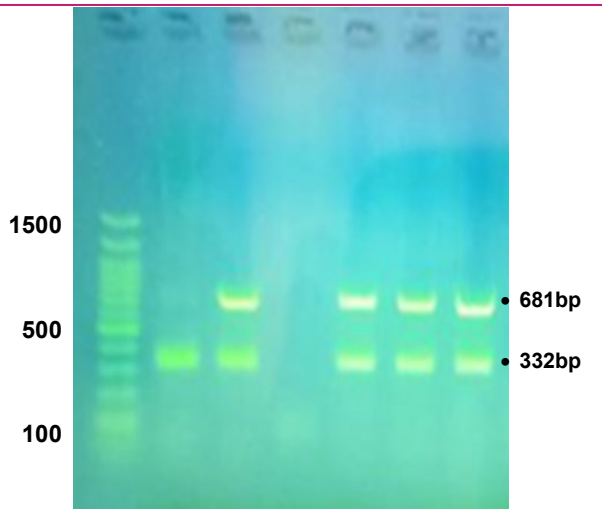

Fig 5. *Multiplex PCR assay for zapA (332bp) and ireA (681bp) detected on P. mirabilis plasmids.*

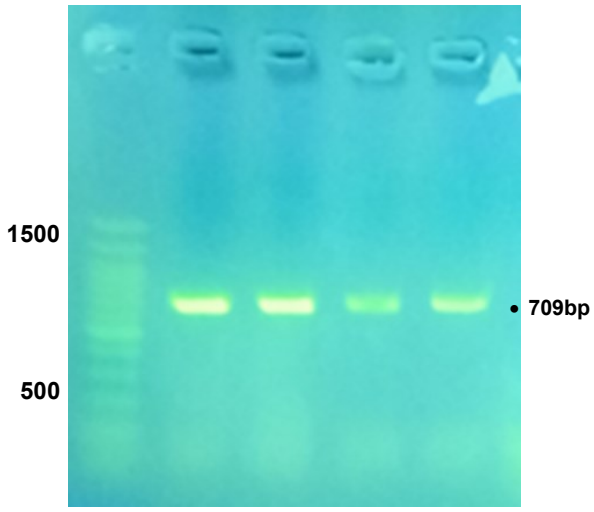

Fig 6. *hpmA gene (709 bp) detected on P. mirabilis plasmids.*

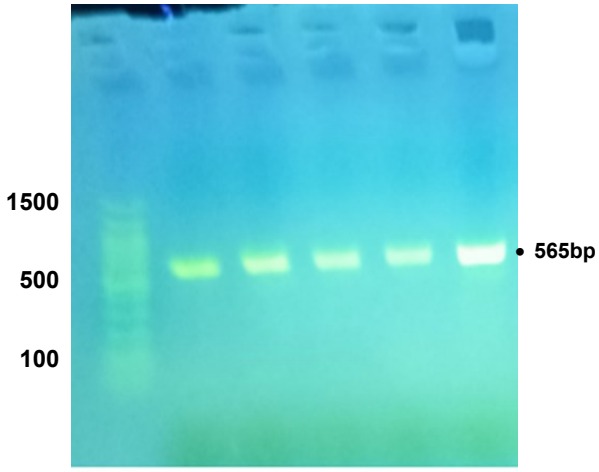

Fig 7. *qnrD gene (565bp) detected on Proteus mirabilis plasmids.*

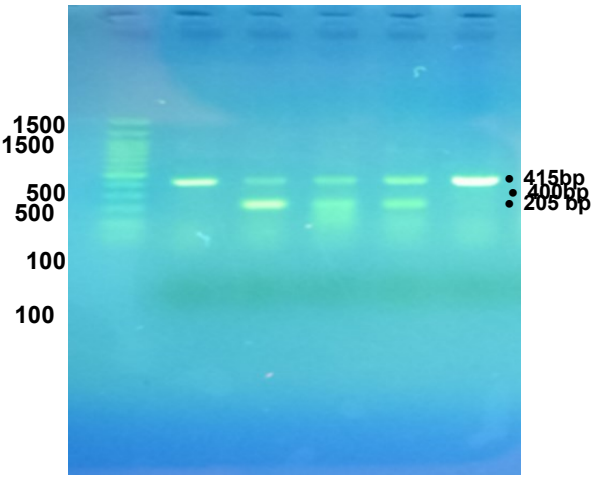

Fig 8. *Multiplex PCR assay for CTXG1 (415bp), G2 (552bp), G9 (205bp), G8 (666 bp) detected on Proteus mirabilis plasmids.*

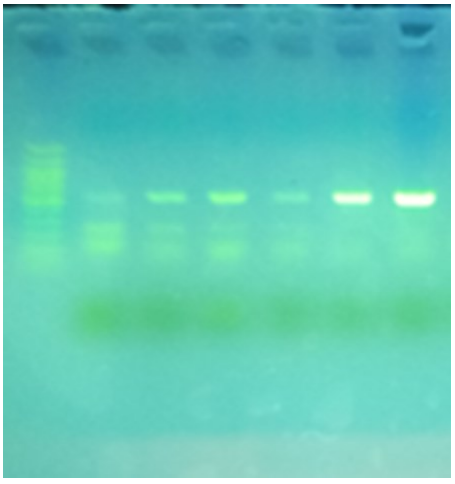

Fig. 9. *Amplicons for Pmlj1 , LJRI gene (400 bp) detected on P. mirabilis plasmids*

2009; Abossedgh *et al*., 2020). Resistance to quinolones in Enterobacteriaceae mainly occurs through point mutations in the quinolone resistance-determining regions (QRDR) of the gyrase and topoisomerase IV genes, resulting in alterations in the drug targets. Additionally, resistance mechanisms involve efflux pump mechanisms. Recently, target protection mechanisms encoded by the nr genes and enzymatic modifications

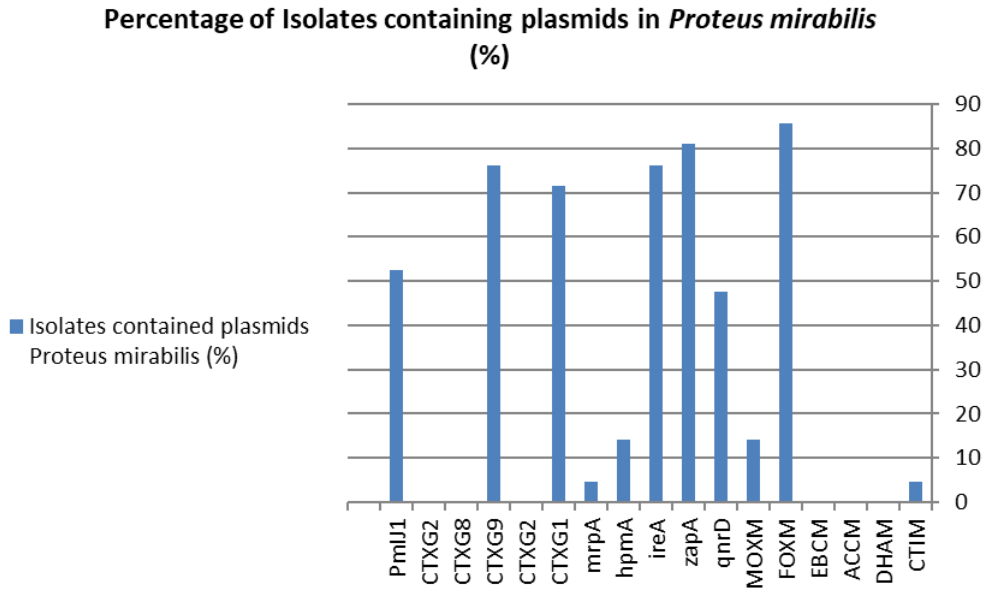

Fig 10. *Distribution of antimicrobial resistance gene in P. mirabilis plasmids*

encoded by ac(6')-Ib-cr have also been identified as contributors to resistance against drugs in this antimicrobial class (Aldred *et al*., 2014). The quinolone resistance gene *qnrD* has been identified as responsible for diminished susceptibility to fluoroquinolones in isolates of *Salmonella enterica* serovar Bovismorbificans and Kentucky strains obtained from humans in China's Henan province. Through complete plasmid sequencing, researchers discovered the novel qnrD gene, which was subsequently cloned alongside both the qnrA1 and qnrS1 genes. These clones were utilized to compare susceptibility patterns in vitro. The novel *qnrD* gene shares similarities with previously described *qnr* genes. This gene encodes a putative pentapeptide repeat protein that confers reduced susceptibility to fluoroquinolones. Recently, Wang and colleagues described another *qnr* gene, *qnrC*, which was found in *P. mirabilis*. This gene encodes a 221-amino acid protein with different amino acid identities from other known *qnrD* (Wang *et al*., 2008).

Conclusion

Bacterial infections have become a major healthcare challenge because of the rise and dissemination of multidrug-resistant bacteria. In recent decades, *Proteus* infections have received special attention because of the emergence of species resistant to various antimicrobial agents, particularly β-lactams. According to the World Health Organization, antimicrobial medication misuse is a universal challenge. This study showed that the percentages of antibiotic resistance and virulence genes located on *P. mirabilis* plasmids are high which indicates these plasmids have becoming more prevalent among clinically isolated *P. mirabilis*, necessitating considerable care to prevent the development of antibiotic resistance. Future work that involves plasmid transformation experiments is sugested to identify the particular phenotypes carried by such plasmids.

Conflict of interest

The authors declare that they have no conflicts of interest.

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