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Molecular Research of The Difference between Chromosome and Plasmid at Harboring Some Virulence and Antibiotic Resistance Genes in *P. Mirabilis*

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ABSTRACT

Background: *Proteus* spp. is gram negative bacteria belonging to the *Enterobacteriaceae* family. *P. mirabilis* is the most commonly isolated species from clinical samples. **Objective:** The present study aimed to investigate and compare the prevalence of virulence genes (*hpmA, hpmB*) and the antibiotic resistant genes ($bla_{\text{CTX-M}}$ and bla_{KPC}) on both chromosome and plasmid in *Proteus mirabilis* isolates. **Material and methods:** A total of 487 samples were collected from different clinical sources. Samples were obtained for patients of different ages were involved of both sexes. The samples were collected from hospitals and private laboratories in Thi-Qar province, Iraq during a period from November 2021 to Abril 2022. A total of the isolates were diagnosed by different laboratory and molecular methods. **Results:** The prevalence of *P.mirabilis* was 8.6 % among collected samples. The findings of virulence genes (*hpmA* and *hpmB*) indicated that 45% of isolates were positive for *hpmA* gene on chromosome and 33.3% on plasmid, while *hpmB* gene rate was 30.9% on chromosome and 16.7% on plasmid. On the other hand the antibiotic resistance genes ($bla_{\text{CTX-M}}$ and bla_{KPC}) test results showed that the $bla_{\text{CTX-M}}$ gene was absent on the chromosome with a very high frequency on the plasmid (95.2%). While the bla_{KPC} appeared in a low rate on the chromosome (38%) but it has been risen on the plasmid (95.2%). **Conclusion**: The examined virulence genes (*hpmA, hpmB*) were found mostly on the chromosome while the antibiotic resistance genes ($bla_{\text{CTX-M}}$, bla_{KPC}) found mostly on the plasmid.

Keywords: P. mirabilis, virulence genes, hemolysins,, antibiotic resistance genes, University of Thi-Qar, Iraq.

.INTRODUCTION

Hauser originally referred to a shape-shifting bacterium he had obtained from putrefied meat as Proteus in bacterial nomenclature in 1885 [(1)]. *Proteus mirabilis*, the motile Gram-negative member of the *Enterobacteriaceae* family, has captivated scientists for many years due to its capacity to develop from short rods into long, multinucleate swarmer cells expressing thousands of flagella [(2)]. Members of the *Proteus* spp are a normal component of the bacterial flora of the intestinal tract. *P. mirabilis* is the most frequently isolated species from clinical samples [(3)]. A vast range of infections are caused by *P. mirabilis*, which has a well-developed array of exoenzymes such as protease, urease, and hemolysins, as well as a high biofilm forming potential [(4)]. *P. mirabilis* coordinates an increase in the synthesis of many virulence factors, such as the haemolysin *hpmA* [(5)]. *hpmB* gene is responsible for activating and transporting of *hpmA* gene, whereas *hpmA* hemolysin is in charge of tissue injury [(6)]. Like other Enterobacterales, Clinical strains of *P. mirabilis* have developed an increased resistance to antimicrobial drugs over the past few decades [(7)]. Plasmids play a significant role for the resistance of *P. mirabilis* to the antimicrobial drugs [(8)]. *Proteus* was susceptible to the β-lactam antibiotics for a long time. Nowadays they are becoming resistant due to the spread of extended-spectrum betalactamase [(9)]. Most frequent ESBLs are the plasmid-mediated CTX-M enzymes [(10)]. Most β -lactam antibiotics are hydrolyzed by the (KPC) enzyme, Numerous KPC variants have been reported, and KPC-producing bacteria have been found all over the world. These bacteria often belong to the order Enterobacterales [(11)]. Bacteria that produce KPC and CTX-M are typically multidrug resistant [(12)]. Bacterial virulence factors might be encoded on chromosomal DNA, plasmids, +any difference between chromosoma and plasmid in harboring these virulence and antibiotic resistance genes in *P.mirabilis* strains.

MATERIALS AND METHODS

Collection of Samples

A total of 487 samples were collected between the 29th of November 2021 and the 20th of April 2022. from different clinical sources, including 316 urine, 63 smears of burns, 20 wound swab, 69 ear swab, and 19 diabetic ulcer swab from both genders and different ages from hospitals and private clinics in Thi-Qar province, Iraq. The samples were transported on Cary Blair swabs and grown on Blood agar and MacConkey agar for 24 hours in an aerobic environment at 37 °C. Microscopic, morphologic, biochemical, and API 20E assays were used to identify the isolated bacteria.

Molecular Analysis

Primers: Research primers are mentioned in **Table 1**.

Extraction of the bacterial DNA: Genomic DNA and Plasmid DNA was extracted and purified according to the instructions of the company Trans/Korea.

Concentration and purity estimation of DNA: The obtained nucleic acids were measured using the Nanodrop spectrophotometer, DNA purity was selected in a wavelength between (260/280 nm), the DNA considered pure when the absorption was 1.8 nm.

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Table1: Sequence of PCR primers and the molecular size of the PCR product.

Proteus strain	Primer	Primers (Sequence (5' – 3'))	Product size (bp)
Proteus spp.	*16S rRNA*	(F) AGAGTTTGATCCTGGCTCAG	1500
		(R) GGTTACCTTGTTACGACTT	
Virulence genes	*hpmA*	(F) GTTGAGGGGCGTTATCAAGAGTC	709
		(R) GATAACTGTTTTGCCCTTTTGTGC	
	hpmB	(F) CAGTGGATTAAGCGCAAATG	422
		(R) CCTTCAATACGTTCAACAAACC	
Antibiotic resistance gene	bla_{CTX}	(F) ACGCTACCCCTGCTATTT-3′	780
		(R) 5′-GCTTTCCGCCTTCTGCTC-3′	
	bla_{KPC}	(F) TGTCACTGTATCGCCGTC	428
		(R) TATTTTTCCGAGATGGGTGAC	

Ethical consent

This study was ethically approved by the Academic and Ethical Committee of Thi-Qar University. Every patient signed an informed written consent for acceptance of participation in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for Social Sciences) version 23 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Qualitative data were represented as frequencies and relative percentages. Chi square test ($\chi 2$) and Fisher's exact test to calculate difference between two or more groups of qualitative variables. Quantitative data were expressed as mean and standard deviation (SD). P value $\leq$0.01 was considered significant.

RESULTS

Out of 487 samples, 45 (9.24%) were identified as *Proteus* sp. 42 (93.75%) were *P.mirabilis,* while 3 (6.25%) belonged to *P.vulgaris*. the infection rate was 52.4% for females and 47.6% for males in the present study patients ages were between (3-81) years. the mean age was 43.2 years.

The molecular study results showed that the hemolysin genes *hpmA* and *hpmB* were present on both plasmid and chromosome. Both genes recorded higher rates on the chromosome 45.2% for *hpmA* and 30.9% for *hpmB.* On plasmid the rates were 30.9% for *hpmA* and 16.7% for *hpmB*. While the antibiotic resistance genes results

showed that the $bla_{CTX\text{-}M}$ gene was totally absent on the Chromosome while the bla_{KPC} gene was found on the chromosome of 45.2% of the *P.mirabilis* isolates. On the other hand both antibiotic resistant genes ($bla_{CTX\text{-}M}$ and bla_{KPC}*)* were found on the plasmid of 95.2% of clinical isolates of *P. mirabilis.*

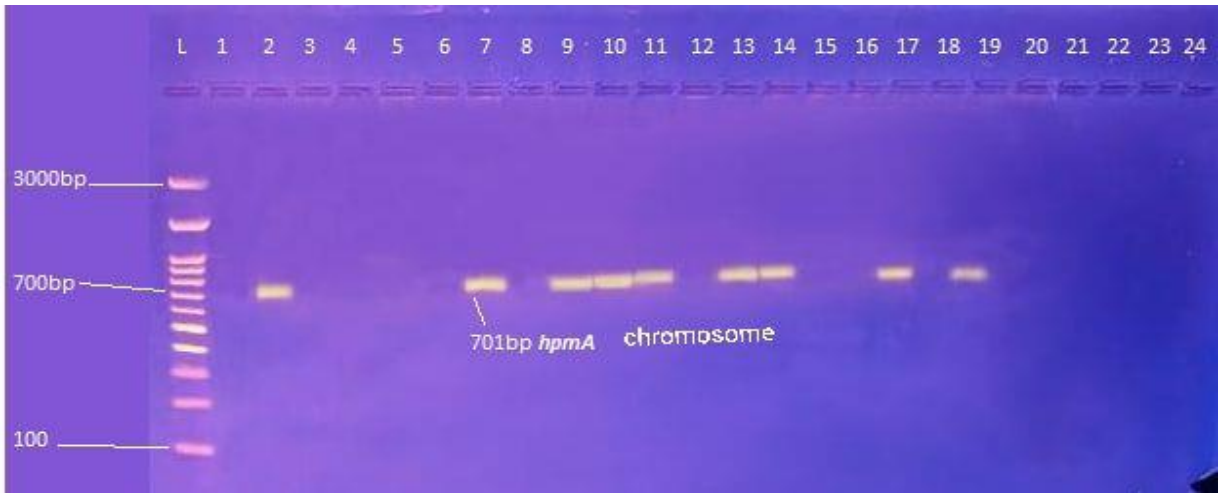

Figure 1: Agarose gel electrophoresis of amplified *hpmA* gene PCR product (701 bp) in chromosome (L: DNA Ladder 100-3000 bp, Agarose: 2%, Volt: 100v, Lanes showing (1, 3, 4, 5, 7, 8, and 19) represent bands of *P.mirabilis* isolates.

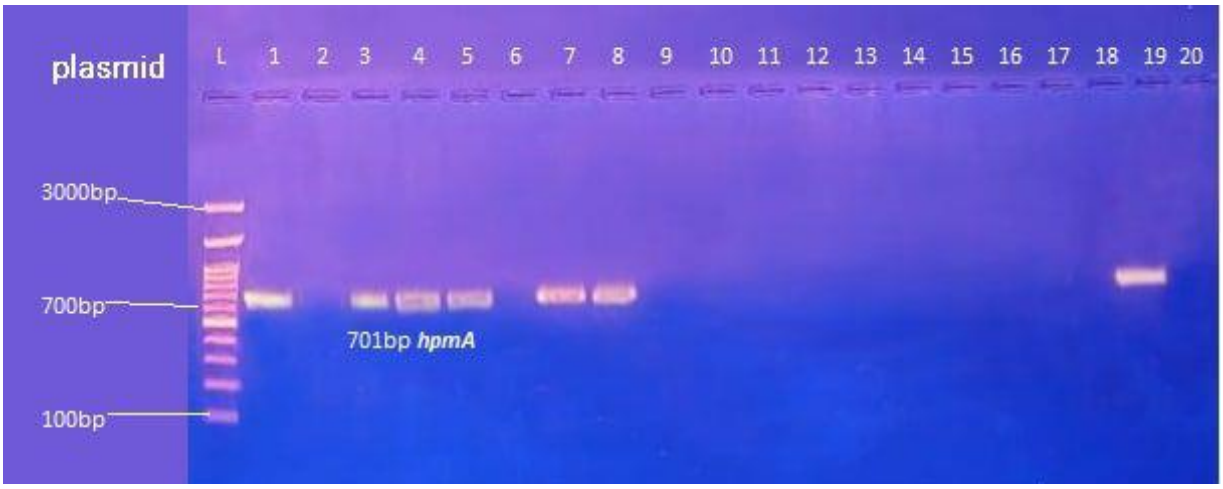

Figure 2: Agarose gel electrophoresis of amplified *hpmA* gene PCR product (701 bp) in plasmid (L: DNA Ladder 100-3000 bop, Agarose: 2%, Volt: 100v, Lanes showing (1, 3, 4, 5, 7, 8, and 19) represent bands of *P.mirabilis* isolates.

Table 1: Comparison of molecular appearance rate of *hpmA* gene in both chromosome and plasmid of *P.mirabilis* isolates from the clinical samples.

Clinical sources	No. of *P. mirabilis* isolates	Frequency and %		X^2	P value
		Chromosome	Plasmid		
Urine	19	8 (42.1)	7(36.8)	0.06	0.01
Burns	9	6(66.7)	4(44.5)	0.40	0.01
wounds	3	0 (0)	1(33.3)	---	0.01
Ear swabs	7	3(42.8)	1(14.3)	1.0	0.01
Diabetic ulcers	4	2(50)	1(25)	0.33	0.01
Total No.	42	19(45.2)	14(33.3)	0.75	0.01
X^2		4.78	10.28	---	
P value		0.01	0.01		

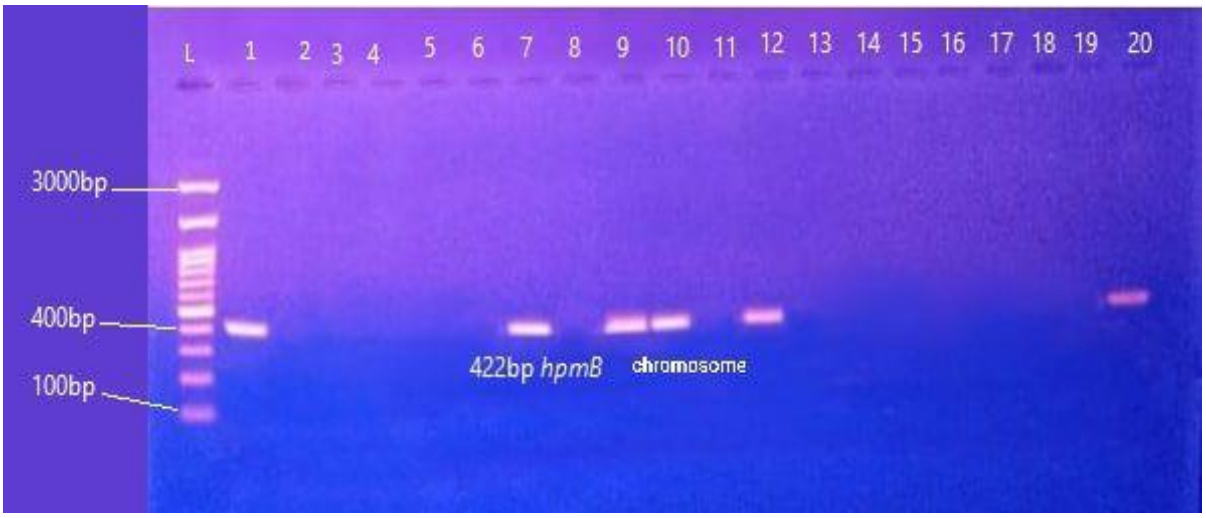

Figure 3: Agarose gel electrophoresis of amplified *hpmB* gene PCR product (422 bp) in chromosome (L: DNA Ladder 100-3000 bop, Agarose: 2%, Volt: 100v, Lanes showing (4, 6, and 20) represent bands of *P.mirabilis* isolates.

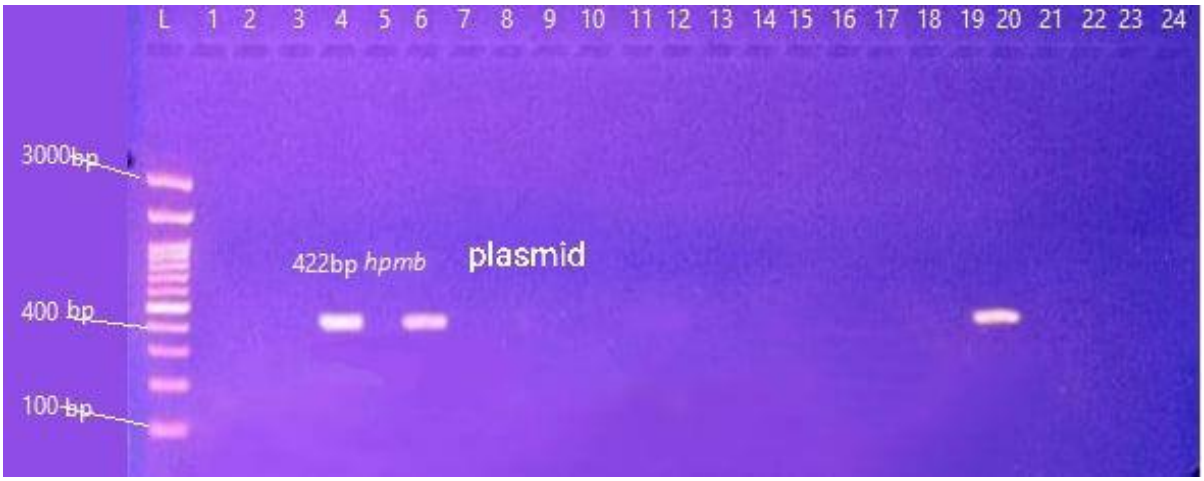

Figure 4: Agarose gel electrophoresis of amplified *hpmB* gene PCR product (422 bp) in plasmid (L: DNA Ladder 100-3000 bp, Agarose: 2%, Volt: 100v, Lanes showing (4, 6, and 20) represent bands of *P. mirabilis* isolates.

Table 2: Comparison of molecular appearance rate of *hpmB* gene in both chromosome and plasmid of *p.mirabilis* isolates from the clinical samples.

Clinical sources	No. of *P. mirabilis* isolates	Frequency and %		X^2	P value
		Chromosome	Plasmid		
Urine	1.5	4(21)	2(10.5)	0.66	0.01
Burns	9	5(55.5)	2(22.2)	1.28	0.01
Wounds	3	0(0.0)	0(0.0)	---	0.01
Ear swabs	8	2(25.0)	1(12.5)	0.33	0.01
Diabetic ulcers	4	2(50.0)	2 (50.0)	0.0	0.01
Total No.	42	13(30.9)	7(16.7)	1.80	0.01
X^2		2.07	0.42	---	
P value		0.01	0.01		

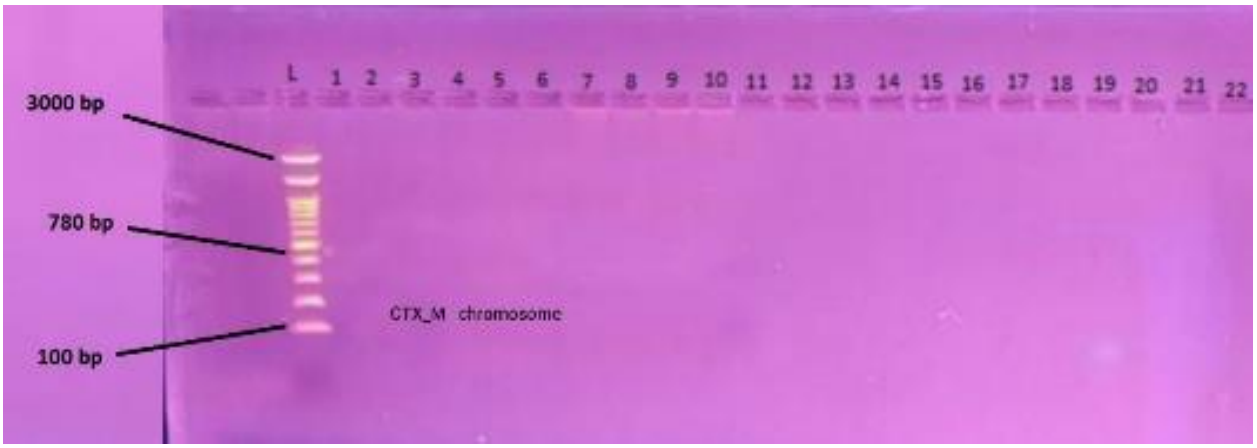

Figure 5: Agarose gel electrophoresis of amplified *bla*$_{CTX-M}$ gene PCR product (422 bp) in chromosome (L: DNA Ladder 100-3000 bp, Agarose: 2%, Volt: 100v, Lanes showing that gene was absent from all samples of *P. mirabilis*.

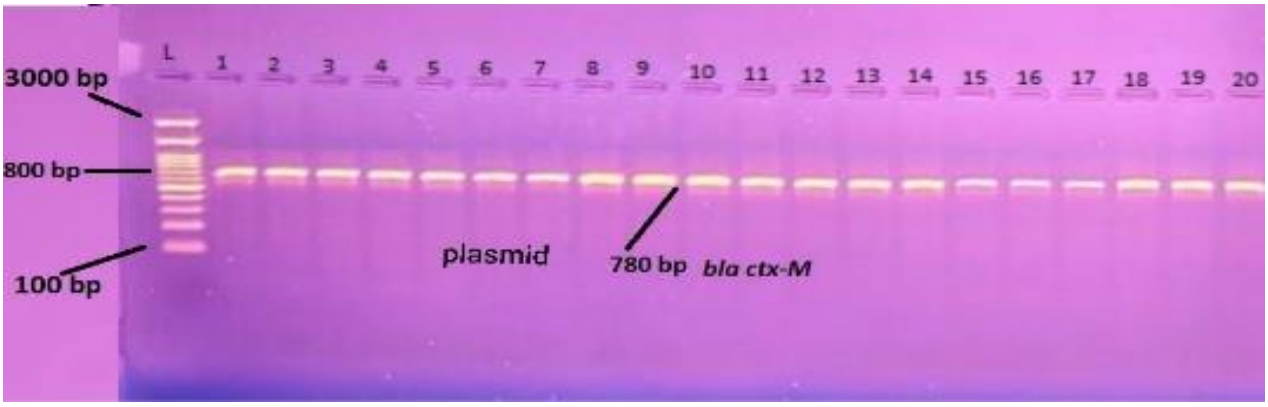

Figure 6: Agarose gel electrophoresis of amplified *bla*$_{CTX-M}$ gene PCR product (422 bp) in plasmid (L: DNA Ladder 100-3000 bp, Agarose: 2%, Volt: 100v, Lanes 1-20 represent bands of *P.mirabilis* isolates.

Table 3: Comparison appearance rate of *bla*$_{CTX-M}$ gene in both chromosome and plasmid of *P.mirabilis* isolates from the clinical samples.

Clinical sources	No. of *P. mirabilis* isolates	Frequency of gene and %		X^2	P value
		Chromosome	Plasmid		
Urine	19	0 (0)	18 (94.7)	0.0	0.01
Burns	9	0 (0)	8 (88.9)	0.0	0.01
wounds	3	0 (0)	3 (100)	0.0	0.01
Ear swabs	7	0 (0)	7 (100)	0.0	0.01
Diabetic ulcers	4	0 (0)	4 (100)	0.0	0.01
Total No.	42	0 (0)	40 (95.2)	0.0	0.01
X^2		0.0	17.75	---	
P value		0.01	0.01		

Figure 8: Agarose gel electrophoresis of amplified *bla*$_{KPC}$ gene PCR product (330 bp) in chromosome (L: DNA Ladder 100-3000 bp, Agarose: 2%, Volt: 100v, Lanes showing (2, 6, 7, 11, 12, 13, 16, and 17) represent bands of *P.mirabilis* isolates.

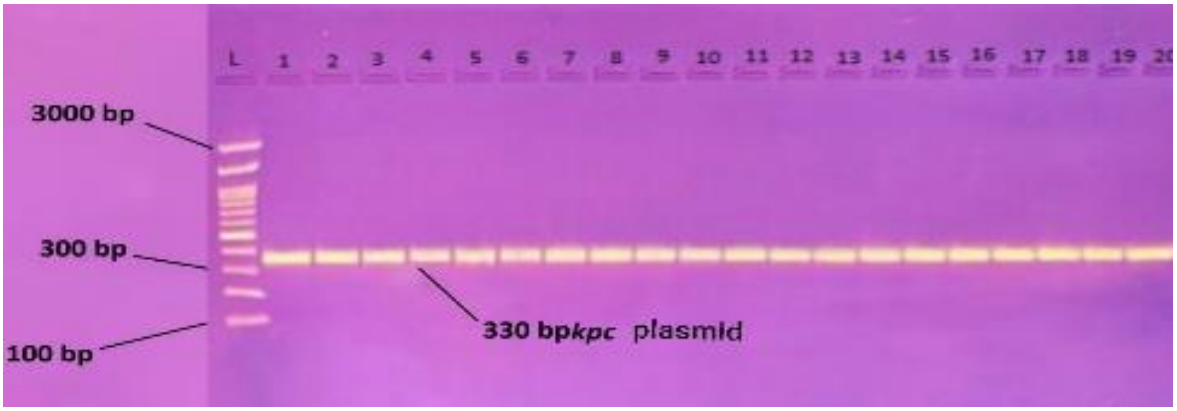

Figure 9: Agarose gel electrophoresis of amplified *bla*$_{KPC}$ gene PCR product (330 bop) in chromosome (L: DNA Ladder 100-3000 bop, Agarose: 2%, Volt: 100v, Lanes 1-20 represent bands of *P.mirabilis* isolates.

Table 4: Comparison appearance rate of *bla*$_{KPC}$ gene in both chromosome and plasmid of *p.mirabilis* isolates from the clinical samples.

Clinical sources	No. of *P. mirabilis* isolates	Frequency and %		X^2	P value
		Chromosome	Plasmid		
Urine	19	6 (31.6)	18 (94.7)	6.0	0.01
Burns	9	5 (55.6)	9 (100)	1.14	0.01
wounds	3	2 (66.7)	3 (100)	0.20	0.01
Ear swabs	7	3 (42.8)	6 (85.7)	1.0	0.01
Diabetic ulcers	4	0 (0.0)	3 (75)	0.0	0.01
Total No.	42	16 (38)	40 (95.2)	10.28	0.01
X^2		2.50	8.90	---	
P value		0.01	0.01		

DISCUSSION

The present study showed that there were no significant differences between males and females at getting the infection with *P. mirabilis* (P value 0.01). our results agreed with [14] who found that female infection rate was 56%, while males infection rate was 43% but disagreed with [15,16,17].

The mean age of the present study was 43.2 years. This agreed with another study [16] from Iran who found that the mean age was 37.7 years. However, it disagreed with a study from china whose finding age was 67.2 years [18].

Haemolysin is a virulence factor produced by *P. mirabilis* which is cytotoxic to epithelial cells [19]. It has been demonstrated that *hpmA* is capable of lysing erythrocytes, bladder epithelial cells, monocytes and B-cell lymphoma cells [20]. While *hpmB* is responsible for activating and transporting *hpmA* [21]. Our results for *hpmA* agreed with other studies which found that the gene frequency was high such as [22,6,23]. However, it disagreed with another study [24].

On the other hand our results for *hpmB* gene disagreed with many other studies. such as studies [6,22] who much higher results for *hpmB* gene frequency. The ESBL gene bla_{CTX} was present on plasmid only in (95.2%) of samples. This finding converged with another study [25] found that (72%) of the isolates were positive for bla_{CTX} gene. But disagreed with [9,26,27] *P.mirabilis* is incapable of producing chromosome borne species-specific β-lactamases, hence the development of resistance in this bacterium depends entirely on the acquisition of plasmid-encoded β -lactamases, particularly the extended-spectrum β -lactamase genes like bla_{CTX} gene, which is encoded for cephalosporins [26,28].

It is clear that the plasmid-mediated CTX-M enzymes originated from *Kluyvera* since the CTX-M genes can be linked to the cefotaximas genes found on chromosomes of *Kluyvera* species [10]. Clinical isolates of *Enterobacteriaceae* commonly have acquired $bla_{CTX\text{-}M}$ genes on conjugative plasmids [29]. The present study showed that all CTX-M genes existed on the plasmid only [28]. Also indicated that Most of the $bla_{CTX\text{-}M}$ genes are harbored by plasmids, whereas a study from Israel found that The genes of $bla_{CTX\text{-}M\text{-}25\ and\text{-}41}$ were also found on the chromosomes of *P.mirabilis* [30].

P. mirabilis carbapenem resistance caused by KPC-2 was first discovered in the United States in 2008 [31]. A study [32] from Brazil found that out of 10 carbapenems and aminoglycosides resistant *P. mirabilis* 9 isolates carried the bla_{KPC} gene. Serine carbapenemases which are known as KPC β-lactamases break down the carbapenems, penicillins, and monobactams more effectively than other ESBLs. Recently, it was shown that KPC-encoding genes were housed in a transposon element that may be transported by plasmids, facilitating mobilization. There have also been reports of clonal spread [12]. A study [11] found that a set of plasmid-borne antibiotic resistance genes complement the chromosomal antibiotic resistance genes in both strains T18 and T21 of the *P. mirabilis.* Notably, plasmids carried the *rmtB,* and $bla_{KPC\text{-}2}$ genes.

In conclusion, the chromosome harbored the virulence genes (*hpmA, hpmB*) in higher rates comparing to the plasmid, while the plasmid harbored the antibiotic resistant genes (bla_{CTX}, $bla_{KPC\text{-}2}$) in much higher rates than chromosome.

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