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DETERMINING THE OCCURRENCE OF SOME VIRULENCE GENES IN PROTEUS SPECIES ISOLATES

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Abstract: Forty isolates of Gram-negative rod-shaped bacteria termed as Proteus, widely known for their swarming motility and urease activity, which cause complicated urinary tract infections (UTIs), were isolated and identified. Two hundred and ten urine specimens collected from the patients suffering from UTIs, who were hospitalized in Babylon hospitals, were used for the isolation of Proteus species isolates. The morphological features (for cells and colonies), biochemical tests, VITEK 2 compact and polymerase chain reaction (PCR) for Proteus-specific genes were used for the identification of these isolates. The assessment of the antimicrobial profiles that represent the prevalence and the level of pathogenicity of the isolates was also carried out. Furthermore, the whole genomic DNA of the isolates was extracted to determine the sites of blaTEM, blaCTX-M, and blaSHV genes on the genome of the isolates.

The results revealed that thirty isolates were P. mirabilis and ten isolates were P. vulgaris. These isolates were given names as PM1 to PM30 for P. mirabilis and PV31 to PV40 for P. vulgaris. The most effective antibiotics against the isolates were erythromycin (97.5%), followed by tobramycin (85%), ampicillin (82.5%), chloramphenicol (60%), piperacillin (55%) and 52.5% for each sulfamethoxazole and azithromycin. The meropenem and imipenem showed less resistance (35%) followed by ciprofloxacin (30%) and gentamicin (15%). The PCR assay exhibited that these isolates carried blaTEM gene at the rate of 38/40 (95%), blaSHV gene at the rate of 33/40 (82.5%) and blaCTX-M gene at the rate of 37/40 (92.5%). Therefore, reducing the frequency and severity of infections, however, more research is needed to understand how the rates of pathogenicity of Proteus species isolates can be controlled.

Keywords: Proteus species, Urinary tract infections, Extended-spectrum β-lactamases, polymerase chain reaction, Virulence genes.

1. Introduction

The Proteus genus is part of the Enterobacteriaceae family [1]. This genus consists of several species, but P. mirabilis and P. vulgaris make up the vast majority of clinical species [1], [2]. The species of the Proteus genus are widely known for spreading UTIs and wound infections. This organism can thrive best in facultative anaerobic environments with average temperatures of around 40°C. P. vulgaris has been connected to urinary tract, wound, burn, bloodstream and respiratory tract infections [3]. Moreover, in one case study, P. vulgaris infection

of the digestive system resulted in bacteremia and brain abscesses [4]. Due to its flagella and the synthesis of adhesions, P. mirabilis can quickly and easily build biofilms that can foul foreign objects like urinary tract catheters [5]. Its capacity to lead to UTIs is connected to its capacity to create biofilms [2].

Additionally, Proteus species are frequently isolated from patient blood, more frequently in connection with a UTI [6]. The ability of Proteus to create biofilms in concert with its capacity to generate bacteremia may signal the potential to cause serious bloodstream infections, such as infective endocarditis (IE), given the high morbidity and mortality that IE brings [7], [8]. Due to its rarity, IE brought on by Gram-negative bacteria poses treatment difficulties because there is insufficient information and no recognized standard of care for treating these illnesses [8]. Many β-lactamases, which are inducible enzymes that are in charge of pathogenic bacteria resistance to β-lactam antibiotics have been examined for their induction processes. P. vulgaris is a useful organism for kinetic investigation of induced β-lactamase synthesis in Gram-negative bacteria because its β-lactamase can be generated by relatively low amounts of β-lactam antibiotics. It explains the kinetics of β-lactamase production by P. vulgaris clinical isolates [9], having various β-lactamase activities.

One of the most significant resistance determinants in Enterobacteriaceae that are developing globally is extended-spectrum β-lactamases (ESBLs) produced by genes of plasmids, which may break down monobactams and cephalosporins with an expanded spectrum. As ESBL-producing bacteria are resistant to the aforementioned drugs and frequently have a multidrug-resistant (MDR) phenotype, including resistance to aminoglycosides and fluoroquinolones, there are just a few effective therapeutic choices left. Morbidity, mortality and medical costs are all linked to infections caused by ESBL production. The representative enzymes of each lineage are designated as the CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25 groups, and at least five distinct lineages of CTX-M-type enzymes have been identified [10]. Hence, this study aimed to determine the role of blaTEM, blaCTX-M, and blaSHV genes in the antimicrobial profile of P. mirabilis and P. vulgaris isolates.

2. Materials and Methods

Collection of Samples: A total of 210 urine specimens were collected from patients suffering from UTIs. These patients, of both sexes and ages, were hospitalized in the hospitals of Babylon (Al-Hillah Teaching Hospital, Mirgian Teaching Hospital and Imam Al-Sadiq Teaching Hospital). The specimens were collected between September 2021 and July 2022.

Isolation and identification: To isolate species of *Proteus*, urine specimens were streaked on blood agar (Himedia), MacConkey agar (Himedia), and UTI chromogenic agar (Himedia), using sterile loops and incubated at 37°C for 24 hours. After incubation time, pure colonies were selected and kept on the nutrient agar (Himedia) at 4°C, for further experiments [3]. The shape, size, texture and colony organization of the colonies were checked with the naked eye, while Gram-stained films were used to examine the cells of a single colony using a 100x oil-emersion light microscope. The isolates were identified depending on the visual traits (for cells and colonies), biochemical tests, VITEK 2 compact and PCR for *the Proteus*-specific gene [11].

Antibiotic Susceptibility Test: Several antibiotic discs (Himedia, India) were used for the assessment of the antimicrobial profiles of the isolates on Muller Hinton medium, which included: piperacillin (100µg), erythromycin (15µg), azithromycin (30µg), imipenem (10µg), tobramycin (30µg), chloramphenicol (30µg), ampicillin (10µg), gentamicin (10µg), meropenem (10µg), sulfamethoxazole (100µg), ciprofloxacin (10µg). According to [12] criteria, *P. mirabilis* and *P. vulgaris* isolates were classified as sensitive or resistant to these antibiotics.
Extraction of DNA: Whole genomic DNA was extracted from *P. mirabilis* and *P. vulgaris* isolates according to the company's instructions (Favorgen, Taiwan).

Primers and Conditions: Cycling thermal programs were used in the reaction of the PCR mixture. The PCR primers used in this study, produced by Macrogen (South Korea), are shown in Table 1.

Table 1. Primers and their conditions used in this study, Macrogen (South Korea).

Primer		Sequence (5----------›3)	Amplicon size (bp)	Conditions (D, A and E)	Cycle No.	Source
TEM	F	GAGTATTCAACATT CCGTGTC	861	94˚C/1 min 57˚C/1 min 72˚C/2 min	35	[13]
	R	TAATCAGTGAGGCACCTATCTC				
SHV	F	AAGATCCACTATCGCCAGCAG	231	94˚C/30 sec 64˚C/1 min 72˚C/2 min	35	
	R	ATTCAGTTCCGTTTCCCAGCGG				
CTX-m	F	GACGATGTCACTGGCTGAGC	499	94˚C/1 min 57˚C/1 min 72˚C/1 min	35	
	R	AGCCGCCGACGCTAATACA				

Abbreviations: D, denaturation; A, annealing; E, extension; F, forward primer; R, Reverse primer.

Composition of PCR Reaction Mixture: According to the manufacturer's instructions, the reaction mixture was carried out in 12 µl of PCR Master Mix (Bioneer, South Korea). The total volume of the reaction was 25 µl and comprised 2 µl from each primer, forward and reverse, 3 µl of DNA, and the volume completed up to 6 µl with free nucleases deionized water (Table 2). A negative control containing all of the above contents without a DNA template was also employed. The amplification reactions were carried out in an automated thermocycler apparatus (Clever Scientific, UK).

Table 2. Composition of reaction mixture.

Content	Volume (µl)
Master Mix	12
DNA Template	3
Forward primer (10 pmol/µl)	2
Reverse primer (10 pmol/µl)	2
Nuclease free water	6
Total volume	25

Electrophoresis: PCR products were run on a 1% horizontal agarose gel stained with red-safe dye for 1 hour at 75 volts. 5 µl of amplification products and 1 µl of loading dye were added to the gel well. The amplified gene electrophoresis fragments were measured using a 100–1500 bp DNA ladder (Promega, USA). According to [14], the gel documentation system (Biometra-Germany) was used to photograph the DNA bands.

Statistical Analysis: The statistical analysis for various parameters was done using Statistical Package for the Social Sciences (SPSS) software version 2010.

3. Results and Discussion

Isolation and Identification: Thirty isolates of *P. mirabilis* and ten of *P. vulgaris* were isolated from 210 urine specimens. These isolates were given names as PM1 to PM30 for *P. mirabilis* and PV31 to PV40 for *P. vulgaris* (Table 3). The identification of the isolates was done depending on the morphological characteristics of each colony and cell, biochemical tests, VITEK 2 compact and PCR of *Proteus*-specific genes. The isolates grew well and showed swarming on blood agar, which caused them to move and generate a thin filmy coating of concentric circles that resemble the ripples left by throwing a rock into a lake. However, on MacConkey agar the isolates didn't swarm and form smooth, pale or colourless colonies.

Table 3. Distribution of *Proteus* isolates with their percentages in collected specimens.

Isolate	No. and the name of the isolate	Percentage of isolate (%)
P. mirabilis	**30 (PM1 to PM30)**	**14**
P. vulgaris	**10 (PV31 to PV40)**	**5**

Antibiotic Susceptibility Test: The sensitivity of forty *Proteus* isolates toward eleven different antibiotics was tested. It was found that most of the isolates possessed multiple resistance to the tested antibiotics. Figure 1, showed that the most effective antibiotics against *P. mirabilis* and *P. vulgaris* isolates were erythromycin (97.5%), followed by tobramycin (85%), ampicillin (82.5%), chloramphenicol (60%), piperacillin (55%) and 52.5% of each sulfamethoxazole and azithromycin. While meropenem and imipenem showed less resistance (35%), followed by ciprofloxacin (30 %) and gentamicin (15%).

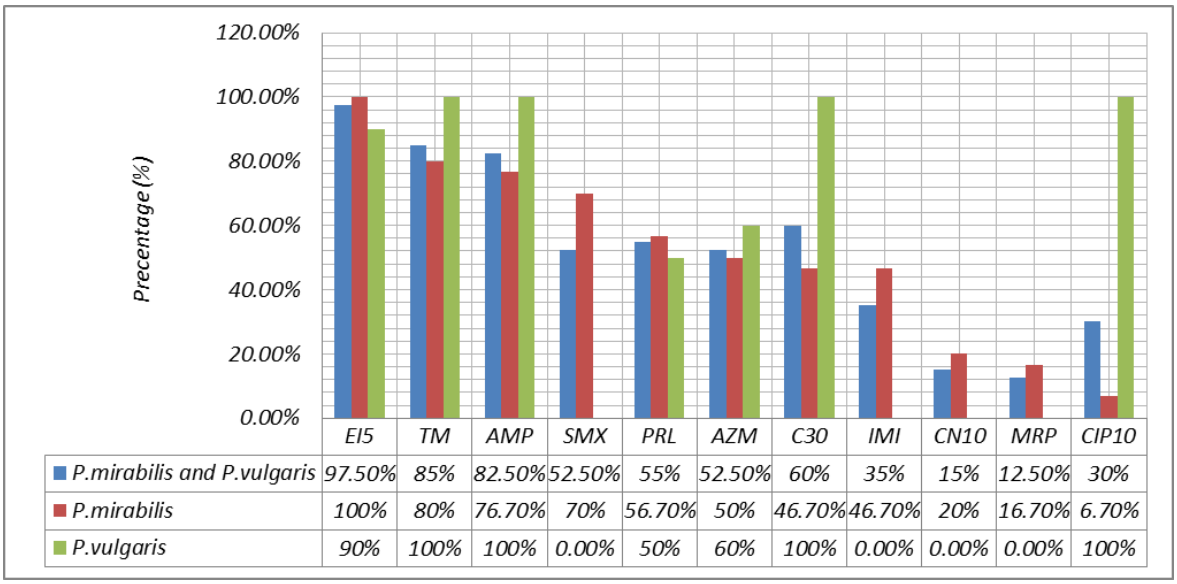

Figure 1. Percentage of antibiotic resistance patterns of *P. mirabilis* and *P. vulgaris* isolates.

Abbreviations: PRL; Piperacillin, E15; Erythromycin, AZM; Azithromycin, IMI; Imipenem, C30; Chloramphenicol, TM; Tobramycin, AMP; Ampicillin, CN10; Gentamicin, MRP; Meropenem, SMX; Sulfamethoxazole and CIP10; Ciprofloxacin.

In a recent study [15] found that 82.5% of *Proteus* isolates were MDR. The rate of resistance of *P. mirabilis* toward ampicillin was (88.3%), piperacillin (72.7%), clindamycin (66.7%), amoxicillin/clavulanic acid (66.2%), and trimethoprim/sulfamethoxazole (50%) is almost exactly in line with the findings reported in [16]. According to [17], [18], gentamycin has a certain effect and *P. mirabilis* had a greater rate of gentamicin resistance (10.3%). The results of the current study agreed with [19] which demonstrated that 94% of *P. vulgaris* isolates were resistant to chloramphenicol, tetracycline and ampicillin, 88% to cefotaxime, 76% to ciprofloxacin, 50% to nitrofurantoin, and 76% to ciprofloxacin. In a study carried out by [20], amoxicillin and penicillin resistance of *Proteus* isolates was found to be 93.3% and 80%, respectively. The types of antibiotics and their frequency of usage in the various patients from whom the specimens were taken may be the cause of the diversity in the resistance pattern of *P. vulgaris* isolates. However, [21] found that all *Proteus* isolates were sensitive to ciprofloxacin whereas [22] revealed that the isolates of *P. vulgaris* were resistant to ciprofloxacin**.** [23] showed that (80%) of *Proteus* isolates were resistant to amoxicillin and 93.3% of them were resistant to penicillin, they also found that a multidrug-resistant trait is present in 84.6% of *Proteus* isolates.

PCR Assay: DNA amplification was accomplished by the thermo-cycler apparatus under optimal conditions using specific primers of *P. mirabilis* and *P. vulgaris* isolates. PCR assay revealed that the isolates carried

the genes of *bla*$_{TEM}$ at the rate of 38/40 (95%), *bla*$_{SHV}$ at the rate of 33/40 (82.5%), and *bla* $_{CTX-M}$ at the rate of 37/40 (92.5%) as shown in Tables 4. Thirty-three isolates (82.5%) carried all these genes. *bla* $_{TEM}$ *and bla* $_{CTX-M}$ genes were found in 4 (10%) isolates (PM2, PM15, PM25, and PV40), *bla*$_{TEM}$ gene was present in just one (PM 2) isolate (2.5%), and these genes were absent in two (PM8 and PM19) isolates (5%).

Table 4. Distribution of gene groups in *P. mirabilis* and *P. vulgaris* isolates.

Isolate No.	*TEM* gene	*SHV* gene	*CTX-M* gene	Genotype
PM1	+	+	+	*TEM, SHV* and *CTX-M*
PM2	+	-	+	*TEM* and *CTX-M*
PM 3	+	+	+	*TEM, SHV* and *CTX-M*
PM 4	+	+	+	*TEM, SHV* and *CTX-M*
PM 5	+	+	+	*TEM, SHV* and *CTX-M*
PM 6	+	+	+	*TEM, SHV* and *CTX-M*
PM 7	+	+	+	*TEM, SHV* and *CTX-M*
PM 8	-	-	-	None
PM 9	+	+	+	*TEM, SHV* and *CTX-M*
PM 10	+	+	+	TEM, SHV and *CTX-M*
PM 11	+	+	+	*TEM, SHV* and *CTX-M*
PM 12	+	+	+	*TEM, SHV* and *CTX-M*
PM 13	+	+	+	*TEM, SHV* and *CTX-M*
PM 14	+	+	+	*TEM, SHV* and *CTX-M*
PM 15	+Ve	-Ve	+Ve	*TEM* and *CTX-M*
PM 16	+	+	+	*TEM, SHV* and *CTX-M*
PM 17	+	+	+	*TEM, SHV* and *CTX-M*
PM 18	+	+	+	*TEM, SHV* and *CTX-M*
PM 19	-	-	-	None
PM 20	+	+	+	*TEM, SHV* and *CTX-M*
PM 21	+	+	+	*TEM, SHV* and *CTX-M*
PM 22	+	+	+	*TEM, SHV* and *CTX-M*
PM 23	+	+	+	*TEM, SHV* and *CTX-M*
PM 24	+	+	+	*TEM, SHV* and *CTX-M*
PM 25	+	-	+	*TEM* and *CTX-M*
PM 26	+	+	+	*TEM, SHV* and *CTX-M*
PM 27	+	+	+	*TEM, SHV* and *CTX-M*
PM 28	+	+	+	*TEM, SHV* and *CTX-M*
PM 29	+	+	+	*TEM, SHV* and *CTX-M*
PM 30	+	+	+	*TEM, SHV* and *CTX-M*
PV 31	+	+	+	*TEM, SHV* and *CTX-M*
PV 32	+	-	-	*TEM*
PV 33	+	+	+	*TEM, SHV* and *CTX-M*
PV 34	+	+	+	*TEM, SHV* and *CTX-M*
PV 35	+	+	+	*TEM, SHV* and *CTX-M*
PV 36	+	+	+	*TEM, SHV* and *CTX-M*

PV 37	+	+	+	*TEM, SHV* and *CTX-M*
PV 38	+	+	+	*TEM, SHV* and *CTX-M*
PV 39	+	+	+	*TEM, SHV* and *CTX-M*
PV 40	+	-	+	*TEM* and *CTX-M*

PCR analysis revealed that there was a single band (499 bp) of the target sequence for *the bla*$_{CTX-M}$ gene of *P. mirabilis* and *P. vulgaris* isolates, except for two isolates (PM 8 and PM19), as shown in Figures 2 and 3.

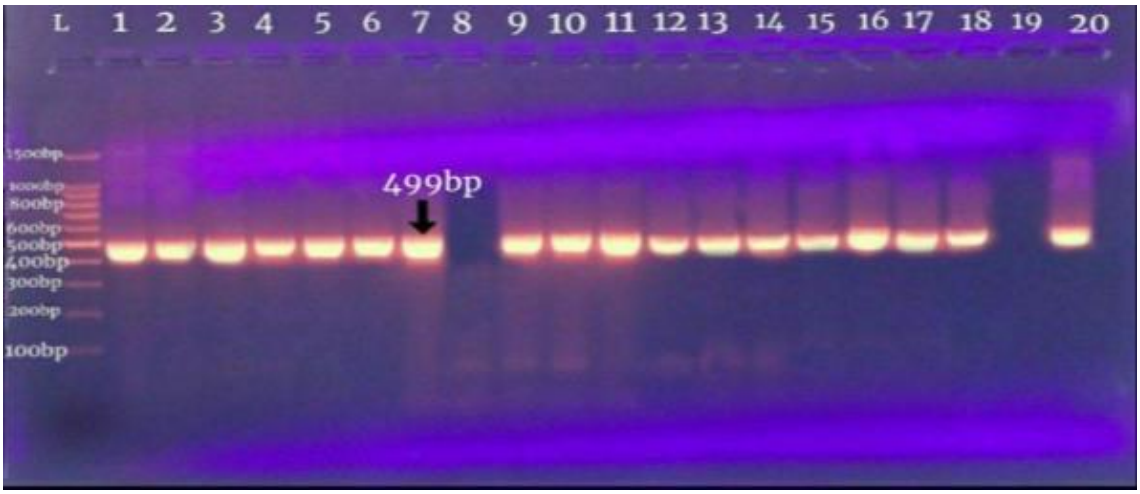

Figure 2. Electrophoresis of PCR products of bla$_{CTX-M1}$ gene of P. mirabilis isolates. The products were separated in 1% agarose gel at 75 V\Cm for 80 min. Lane L: DNA ladder (1500 bp), Lanes 1-20: the isolates PM1 to PM 20, represent the positive results (499 bp), except PM8 and PM19 isolates that represent the negative results.

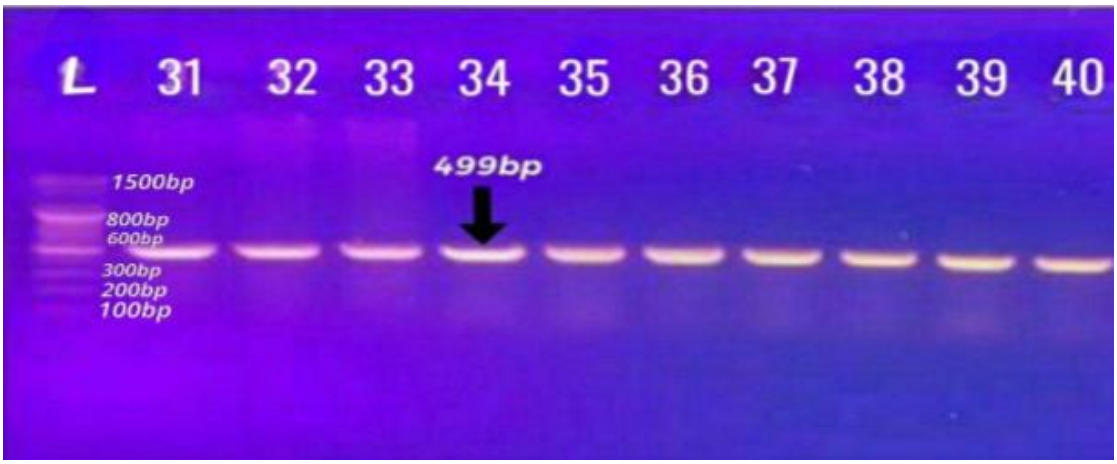

Figure 3. Electrophoresis of PCR products of the bla$_{CTX-M1}$ gene of P. vulgaris isolates. The products were separated in 1% agarose gel at 75 V\Cm for 80 min. Lane L: DNA ladder (1500 bp), Lanes 31-40: the isolates PV31 to PV40 represent the positive results (499 bp).

The class A ESBLs known as *CTX-M*-type enzymes are quickly gaining popularity among Enterobacteriaceae in all parts of the world. Serine β-lactamases, or ESBLs are categorized as class A by Ambler's molecular and structural classification system**.** The majority of ESBLs generated by Enterobacteriaceae members are now *CTX-M* enzymes [24]. Since 1995 [25], when Toho-1, now known as *CTX-M-44* was discovered in Japan, ESBLs frequently contain *CTX-M* enzymes. All 71 clinical isolates of *P. mirabilis* strain that produced *CTX-M* and were gathered in Japan between 2001 and 2003 had *bla* $_{CTX-M-2}$ gene [26]. In the current study, PCR analysis revealed

that a single band (231 bp) of the target sequence of *the bla*$_{SHV}$ gene was found in most isolates of *P. mirabilis* and *P. vulgaris* isolates, except the isolates PM2, PM8, PM15, PM19, PV32 and PV40, as shown in Figures 4 and 5.

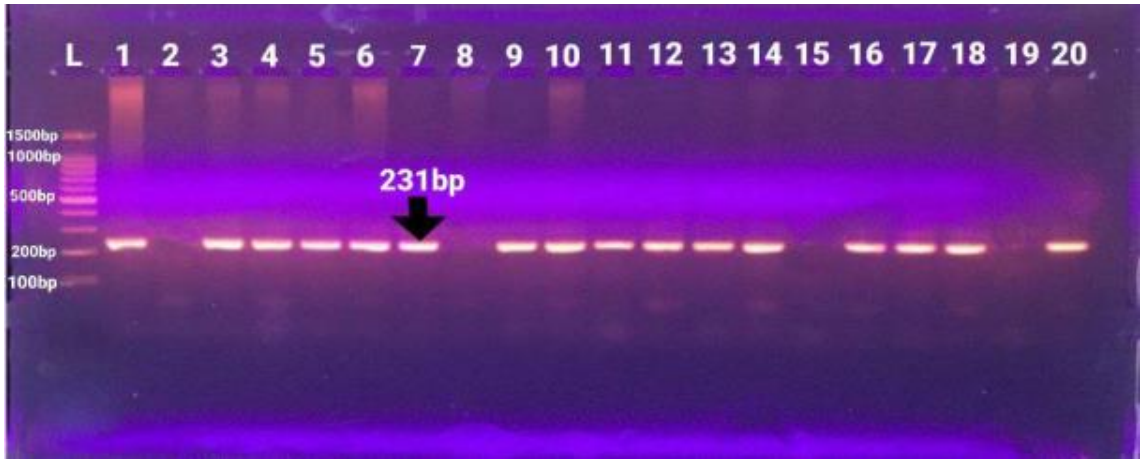

Figure 4. Agarose gel electrophoresis of PCR products of bla$_{SHV}$ gene of P. mirabilis isolates. The products were separated in 1% agarose gel at 75 V\Cm for 80 min. Lane L: DNA ladder (1500 bp), Lanes 1-20: the isolates PM1 to PM20, except PM2, PM8, PM15 and PM19 isolates, represent the positive results (231 bp).

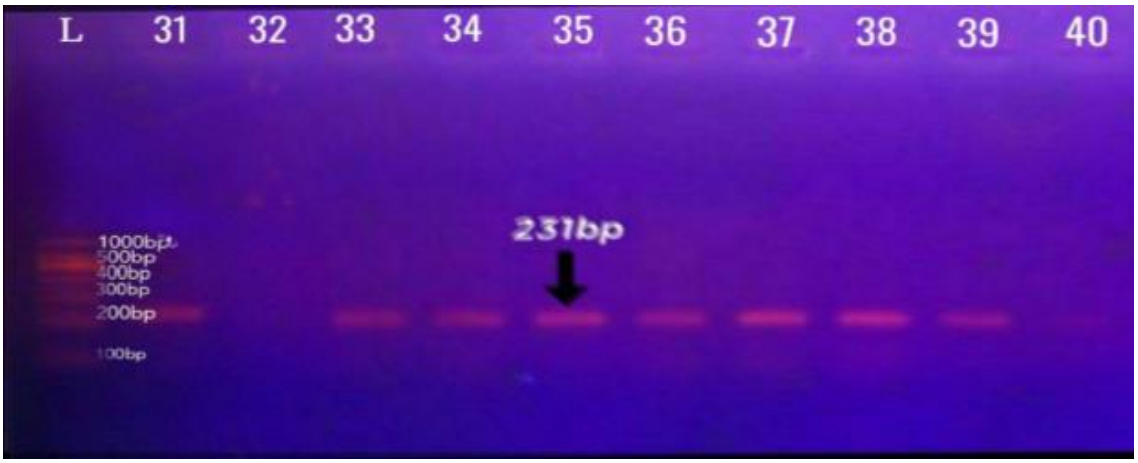

Figure 5. Electrophoresis of PCR products of bla$_{SHV}$ gene of P. vulgaris isolates. The products were separated in 1% agarose gel at 75 V\Cm for 80 min. Lane L: DNA ladder (1500 bp), Lanes 31-40: the isolates PV31 to PV 40, except the isolates PV32 and PV40, represent the positive results (231 bp).

The presence of ESBLs of *the SHV* family on self-transmissible plasmids illustrates the mobility of this class of enzymes. The other beta-lactamase is referred to as *SHV* because of its variable sulfhydryl active site. ESBL-producing bacteria are becoming well known, they cause different diseases, including cholangitis, UTIs and intra-abdominal infections. Everywhere, but especially in the US, Asia, and Europe, *Proteus* species that produce ESBLs are becoming more prevalent [27]. In [28], reported that *bla*$_{TEM}$ and *bla*$_{SHV}$ genes prevalence rates were 60% and 23.3%, respectively. The result of PCR analysis of this study referred to the presence of a single band (861bp) of the target sequence of *the bla*$_{TEM}$ gene of *P. mirabilis* and *P. vulgaris* isolates, except PM8, PM19 and PV32 isolates, as shown in Figures 6 and 7.

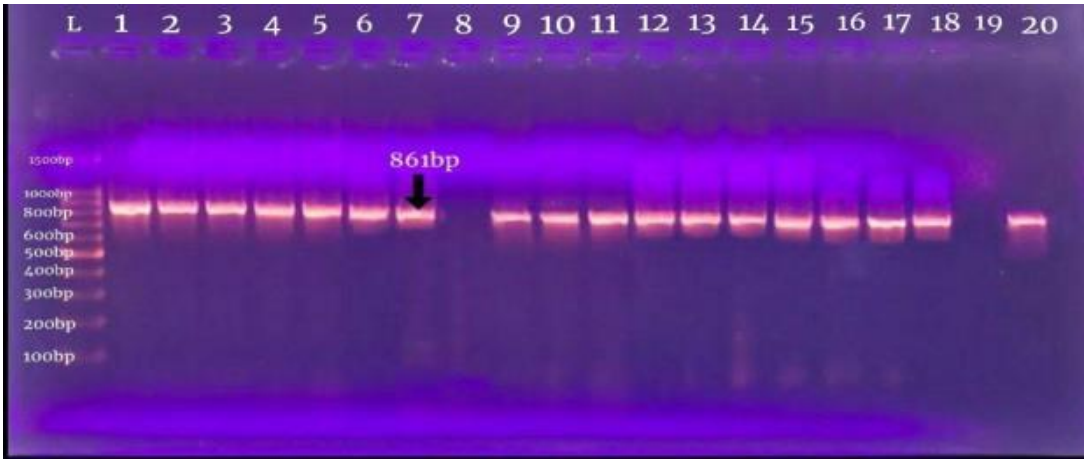

Figure 6. Electrophoresis of PCR products of bla$_{TEM}$ gene of P. mirabilis isolates. The products were separated in 1% agarose gel at 75 V\Cm for 80 min. Lane L: DNA ladder (1500 bp), Lanes 1-20: the isolates PM1 to PM 20, except the isolates PM8 and PM19, represent the positive results (861 bp).

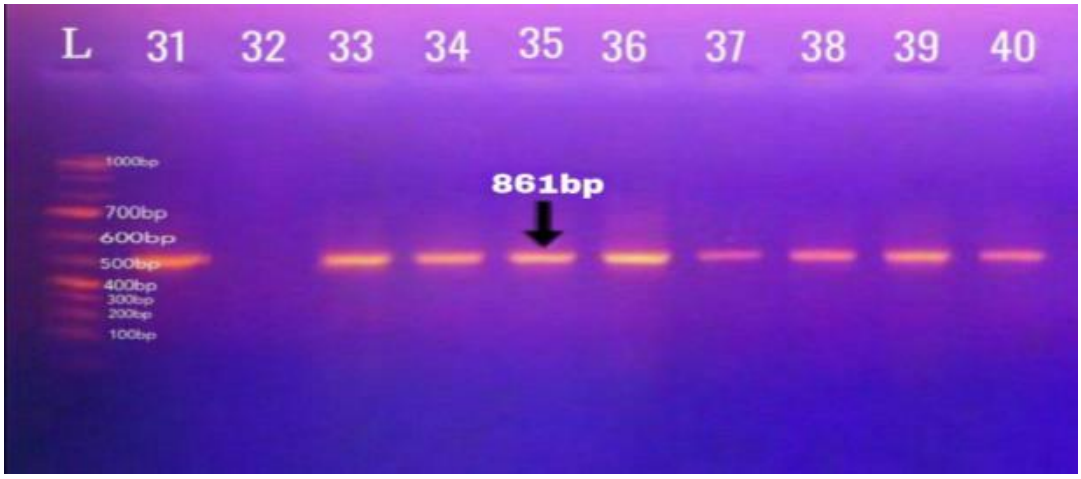

Figure 7. Electrophoresis of PCR products of bla$_{TEM}$ gene of P. mirabilis isolates. The products were separated in 1% agarose gel at 75 V\Cm for 80 min. Lane L: DNA ladder (1500 bp), Lanes 31-40: the isolates PV31 to PV40, except the isolates PV32, represent the positive results (861 bp).

Due to the synthesis of the *TEM* and *CTX-M* genes, *P. mirabilis* BDUMS1 (*KY617768*) and *Escherichia coli* BDUMS3 (*KY617770*), in particular, demonstrated a significant prevalence rate of resistance, the findings led to the conclusion that UTIs are more likely to increase antibiotic resistance [29]. ESBL is a mediator of *Proteus* improved resistance to β-lactam medicines. According to [21], integrons, transposons, and horizontal gene transfer may be to blame for this rise in antibiotic resistance. The capacity of *Proteus* to hydrolyze broad spectrum β-lactamase antibiotics and inhibition by β-lactamase inhibitors, particularly clavulanate, define them biochemically. *P. mirabilis* and *P. vulgaris* isolates carried one resistance gene that implemented strong resistance to ampicillin and tobramycin, while *bla* $_{TEM}$ and *bla* $_{CTX-M}$ genes its appearance excellent resistance to four antibiotics (piperacillin, erythromycin, chloramphenicol and tobramycin) and the *bla* $_{TEM}$ gene is also appear resistance to ampicillin, chloramphenicol and tobramycin, Table 5 displays this outcome. The occurrence of the β-lactamase genes is extremely significant and is a cause for health concern. By destroying the β-lactam ring in medicines, this enzyme helps bacteria develop resistance to common β-lactam ring antibiotics. According to [30], *bla*$_{TEM}$, *bla*$_{SHV}$, and *bla*$_{CTX-M}$ genes were related to six common β-lactamase resistance genes in *Proteus* species.

Concerns regarding the reliance on the increased variety of concerns regarding the usage of β-lactam medications and the advent of pan-resistant pathogens due to the production of β-lactamases by isolates of the Enterobacteriaceae [31]. *Proteus* possesses an inherent resistance to cephalosporin and ampicillin because of an

extended spectrum of β-lactamase [32]. Chromosome-encoded class C β-lactamases may be expressed to cause resistance to expanded spectrum cephalosporins [21]. The global occurrence of antibiotic-resistant infections is mostly caused by integrons, transposons, and R plasmids, which all play a role in horizontal gene transmission. *Klebsiella pneumoniae* and *E. coli*, two possible ESBL species, have a high ability to carry and transfer *bla* genes, according to earlier research, which poses a threat to human health [33]. The treatment is rendered ineffective by *MDR P. mirabilis*, and novel, forward-thinking methods are required to combat the problem. The pharmacological targets will help researchers create new defenses against the MDR *P. mirabilis* [34].

Table 5. Pattern of *P. mirabilis* and *P .vulgaris* isolates to different antibiotics.

Antibiotic	*TEM, SHV* and *CTX-M*		*TEM* and *CTX-M*		*TEM*		None	
	No.	%	No.	%	No.	%	N.	%
PRL	17	51.5	4	100	1	1	0	0
E15	26	78.8	4	100	0	0	0	0
AZM	19	57.6	1	25	0	0	1	50
IMI	13	39.4	1	25	0	0	0	0
TM30	7	21	1	25	1	100	0	0
C30	18	54.5	4	100	1	100	1	50
AMP	28	84.8	3	75	1	100	1	50
CN10	5	15	1	33	0	0	0	0
MRP	5	15	0	0	0	0	0	0
SMX	25	75.8	3	75	0	0	2	100
CIP10	10	30	1	25	1	100	0	0

Abbreviations: PRL; Piperacillin, E15; Erythromycin, AZM; Azithromycin, IMI; Imipenem, TM30; Tobramycin, C30; Chloramphenicol, AMP; Ampicillin, CN10; Gentamicin, MRP; Meropenem, SMX; Sulfamethoxazole and CIP10; Ciprofloxacin.

Diabetes patients' feet, the respiratory system, the urinary system, burns, wounds, and many other illnesses are all caused by *P. mirabilis*. Because of its strong antimicrobial resistance, this infection requires new therapeutic approaches to be defeated. One method for treating MDR *P. mirabilis* infections, particularly biofilm-based illnesses, is the use of bacteriophages [35]. Highly resistant infections may be released as a result of several events, including the misuse of antibiotics and the dissemination of clonally resistant germs. Among the drug resistance mechanisms, ESBLs are crucial in the development of resistance to widely used antibiotics like cephalosporins and penicillins. ESBL genes can further contribute to an increase in drug resistance, including in MDR isolates, as a result of the extensive dissemination of pathogens in the community through plasmids and integrons. Overall, the data show that *P. mirabilis* and *P. vulgaris* isolates are continuously developing resistance to both β-lactams and other classes of antibiotics [36], [37]. Research has shown that the genes encoding in examined bacteria, *CTX-M* and *TEM* β-lactamases predominate over genes generating *SHV*-type β-lactamases that have not been detected. The findings of this study are consistent with those of earlier investigations [38].

4. Conclusion

The burden of infectious diseases in Iraq has increased due to the predominance of bacteria that produce ESBLs, where UTIs are a major public health concern. Therefore, $bla_{CTX\text{-}M}$ and bla_{TEM} genes are the most abundant in *Proteus* species isolates that produce ESBLs. Since bacteria have developed resistance to almost all of the classes of antibiotics currently in use, treating bacterial infections is challenging. Therefore, *P. mirabilis* and *P. vulgaris* isolates pose significant issues that affect people all over the world. The high prevalence of resistance genes is a

risk factor that lengthens the healing process and, in extreme situations, may be lethal. Hence, there is a critical need for research in the area of drug resistance since it will enable us to keep track of the prevalence of resistant bacteria and, as a result, better recommendations on how to use antibiotics to treat bacterial illnesses.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

H. F. Naji; methodology, writing—original draft preparation, A. A. Hassan; writing—review and editing, A. A. Hassan; paraphrasing. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement:

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Data Availability Statement:

No Data Availability Statement.

Conflicts of Interest:

The authors declare no conflict of interest.

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