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Research Article

Molecular Study Of MDR *P.mirabilis* Genes Isolates From different clinical Infections

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Abstract

In this study, The patients specimens of different infections were obtained from hospitals and specialized centers in Najaf province, a total of specimens 120 (100%), which included urine specimens 60 (50%), burn swabs 15 (12.5%), wound infections 15 (12.5%), ear swabs 15 (12.5%), Blood infections 15 (12.5%). Out of 120, only 95 (79.16%) were considered positive growth and 25 (20.83%) showed non- bacterial growth, On the other hand, *P. mirabilis* isolates where found in different clinical cases included 55 (57.89%) isolates in urine, 16 (16.84 %) isolates in burn swabs, 9 (9.47%) isolates in wound infections, 8 (8.42%) isolates in ear swabs, 7 (7.36%) isolates from the blood, From October 2024 to January 2025. Specimens were taken from patients hospitalized in Al-Hakim General Hospital, Al-Sadr Medical City, and the Burn Center in Najaf Al-Ashraf, and they were transferred to the Microbiology Laborator in the Biology Department in the Faculty of Science. The results showed that *P. mirabilis* isolates has a great resistance to most commonly antibiotics used in treatment of different infections, the highest rate of resistance is seen with Tetracycline 60/60 (100%) followed by Doxycycline 59/60 (98%), Gentamycin 58/60 (96%), Nalidixic Acid 58/60 (96%), Tobramycin 54/60 (90%), Ceftazidime 54/60 (90%), Ciprofloxacin 52/60 (86%), Trimethoprime- sulfamethaxazol 52/60 (86%), Amoxicillin- clavulanic 48/60 (80%), Cefotaxime 45/60 (75%), Ceftriaxone 45/60 (75%) whereas the low rate of *P. mirabilis* within Impenem 6/60 (10%), Meropenem 2/60 (3%), Amikacin 0/60 (0%). The results showed that the capacity of some *P. mirabilis* isolates to biofilm formation, out of 60 (100%) isolates of *P. mirabilis* only 45 (75%) were give positive results with biofilm formation and appear black dry crystalline colonies on the CRA plates and 15 (25%) were non - biofilm producers when the colonies of *P. mirabilis* producer remained pink or red colored, The results showed that the *sul1* gene was detected in *P. mirabilis* isolates , out of 26 (100%) isolates only 20 (77 %) were have *sul1* gene , The results also showed that the *(GyrA)* gene was detected in *P. mirabilis* which were in 25 (96%) isolates , the results display 5 (19%) isolates were have *Ant (2)-1a* gene, Regarding *SHV* gene was detected in 8(30%) of *P. mirabilis* isolates, while the results showed that the *rmtB* gene 24 (92v%) of *P. mirabilis* isolates.

1. Introduction

P. Mirabilis is part of the *Enterobacteriaceae* family of bacilli, is a gram-negative, facultative anaerobe with an ability to ferment maltose and inability to ferment lactose, *P. mirabilis* also has swarming motility and the ability to self-elongate and secrete a polysaccharide when in contact with solid surfaces ; this allows for attachment and easy motility along surfaces (e.g., medical equipment), the flagella of *P.*

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mirabilis are what allow for its motility ; not only does this help support colonization, but it also has been associated with its ability to form biofilms and is suggested to contribute to resistance to host defenses and certain antibiotics [1]. Antibiotic resistance was reported to occur when a drug loses its ability to inhibit bacterial growth effectively, bacteria become 'resistant' and continue to multiply in the presence of therapeutic levels of the antibiotics, bacteria when replicates even in the presence of the antibiotics, are called resistant bacteria, antibiotics are usually effective against them, but when the microbes become less sensitive or resistant, it requires a higher than the normal concentration of the same drug to have an effect [2]. The development of specific mechanisms of resistance had provoked their therapeutic use, several *Enterobacteriaceae* strains have been isolated whichare resistant to antibiotics, Gram-negative bacteria are intrinsically resistant to several antibiotic classes because of the presence of a second, OM comparedto Gram-positive bacteria which these antibiotics cannot penetrate [3].

2. Materials and Methods

2.1. Samples Collection and Bacterial Identification

A total number of 120 were collected and worked on it from patients with different clinical cases (such as, otitis media, UTI , burn infections, wound and blood) admitted to center for reaserch in AL-Najaf Governorate, during the period from (October 2024 to January 2025). All specimens were collected in a way to avoid any possible contamination, and the specimens were taken and closed until they were transferred to the Advanced Microbiology Laboratory / Faculty of Science / University of Kufa and cultured on different media for 24 h at a temperature of 37C°for bacterial diagnosis.

2.2. Antibiotic Susceptibility Test For *P. Mirabilis* Isolates

Muller Hinton agar was prepared, it is sterilized in the autoclave and poured in petri dishes, then antibiotic resistance *P.mirabilisisolates* were streaked by sterile swab on petri dish and placed antibodies disc and incubated the dishes at 37°C for 24 h, the diameter of inhibition zones was measured using a meter ruler [4].

2.3. Detection of Biofilm Formation For *P. Mirabilis* Isolates

Biofilm production by isolated from different infection pathogens in our study was detected by microtiter plate method [5].

2.4. Molecular Techniques

Extraction of Genomic DNA

Genomic DNA was extracted by using a method of [6].

Molecular Identification

Gel electrophoresis was used to determine of DNA via UV trans illuminator, the primer was planned by Alpha DNA company, Canada as in Table 1.

Table 1: Primers used in this study.

Primer Type	Primer sequence (5'-3')	Amplicon size (bp)
sul1	F: CGGCGTGGGCTACCTGAACG R: GCCGATCGCGTGAAGTTCCG	432
blaSHV	F: CTTTACTCGCCTTTATCG R: TCCCGCAGATAAATCACCA	827
Ant(2)-1a	F: ACGCCGTGGGTCGATGTTTGAGTG R: ACGCCGTGGGTCGATGTTTGATGT	572
GyrA	F: GGTATACCGTCGCGTACTTT R: CAACGAAATCGACCGTCTCT	311
blaVIM	F: TGCGCTTCGGTCCAGTAGA R:TGACGGGACGTATACAACCAGAT	390
rmtB	F: GCT TTC TGC GGG CGA TGT AA R: ATG CAA TGC CGC GCT CGT AT	173

PCR Thermo - Cycling Conditions

The PCR tubes were placed on the PCR machine and the right PCR cycling program parameters conditions were installed as in Table 2.

Table 2: Amplification conditions of genes were used by PCR reactions.

Gene name	Temperature (C°) / Time					Cycles Number
	Initial Denaturation	Cycling conditions			Final Extension	
		Denaturation	Annealing	Extension		
sul1	94/1 min	98/30 sec	55/30 sec	72/30 sec	72/10 min	30
blaSHV	95/4 min	95/30 sec	45/30 sec	72/1min	72/5 min	35
Ant(2)"-1a	94/5 min	94/30 sec	55/30 sec	72/1min	72/10 min	30
gyrA	95/2 min	95/10 sec	57/30 sec	72/1 min	72/7 min	40
blaVIM	95/5 min	95/30 sec	60/1 min	72/1 min	72/5 min	45
rmtB	94/4 min	94/1 min	50/1 min	72/1.5min	72/5 min	35

3. Results and Discussion

3.1. Distribution of Patient Specimens For Different Infections

The patients specimens of different infections were obtained from hospitals and specialized centers in Najaf province, a total of specimens 120 (100%), which included urine specimens 60 (50%), burn swabs 15 (12.5%), wound infections 15 (12.5%), ear swabs 15 (12.5%), Blood infections 15 (12.5%), as shown Table 3.

Table 3: Number and percentage of specimens collected from different infections.

Type of Specimens	NO. of Specimens	Percentage (%)
Urine Specimens	60	50%
Burn Swabs	15	12.5%
Blood infection	15	12.5%
Otitis media Swabs	15	12.5%
wound infections	15	12.5%
Total	120	100 %

3.2. Identification of *P. Mirabilis* that Isolated From Different Infections

Initial identification of *P. mirabilis* isolates based on morphological characteristics of the colonies on MacConkey agar, Xylose lysine deoxycholate agar (XLD), CHROM agar and blood agar, *Proteus* grows on the Blood agar plate in successive waves to form a thin filmy layer of concentric circles (swarming) and without hemolysis, while in the MacConkey agar medium does not ferment lactose and forms smooth, pale or colorless (NLF) colonies. The colonies of *P. mirabilis* appeared as light brown colonies on CHROM agar and then differentiate these bacteria from *E.coli* colonies, all the isolates were cultured on Xylose lysine deoxycholate agar (XLD), *P. mirabilis* appeared red colony on XLD agar as shown in Figure 1, [7].

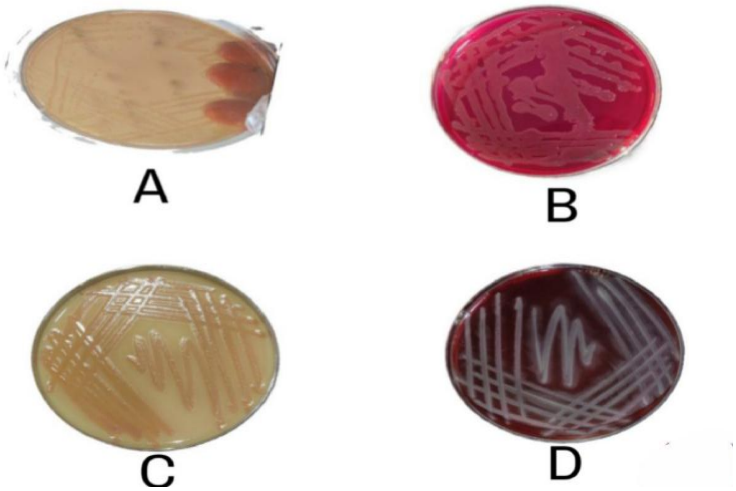

Figure 1: *P. mirabilis* on different culture media (A) MacConkey agar, (B) XLD agar, (c) CHROM agar and (D) Blood agar

Table 4: Antibiotic susceptibility test for 60 *P. mirabilis* isolates.

Antibiotic disk	No.(%) of isolates exhibited	
	Resistance	sensitive
Tetracycline	60/60 (100%)	0/60 (0%)
Doxycycline	59/60 (98%)	1/60 (1%)
Gentamycin	58/60 (96%)	2/60 (3%)
Nalidixic Acid	58/60 (96%)	2/60 (3%)
Tobramycin	54/60 (90%)	6/60 (10%)
Ceftazidime	54/60 (90%)	6/60 (10%)
Ceftriaxone	45/60 (75%)	15/60 (25%)
Ciprofloxacin	52/60 (86%)	8/60 (13%)
Trimethoprime- sulfamethaxazol	52/60 (86%)	8/60 (13%)
Amoxicillin- clavulanic	48/60 (80%)	12/60 (20%)
Cefotaxime	45/60 (75%)	15/60 (25%)
Impenem	6/60 (10%)	54/60 (90%)
Meropenem	2/60 (3%)	58/60 (96%)
Amikacin	0/60 (0%)	60/60 (100%)

3.3. Antibiotics Susceptibility Of *P. Mirabilis* Isolates

Because of the *P. mirabilis* is most frequent than the other bacterial species, antibacterial susceptibility test was conducted for 60 resist *P. mirabilis* isolates against 14 commonly used antibacterial agents by using the disk diffusion method, the results were interpreted according to the diameter of inhibition zone and compared with stander zones of inhibition determined by [8].

Misuse and overuse of antibiotics by healthcare professionals and the general public are among the many factors contributing to an increase in antimicrobial resistance rates [9]. Inadequate surveillance systems and dependence on reliable microbiological techniques also contribute to the improper prescription of antibiotics [10]. According to [11], *P. mirabilis* produces ESBLs, carbapenamase, and AmpC as part of its antibiotic resistance mechanism. These enzymes pose a high risk to the public and are responsible for numerous outbreaks. ESBLs are also expensive, increase the length of hospital stays, and cause more complications. Our results suggest that those antibiotics should not be used in treatment of *P. mirabilis* infections, as it will lead to failure of therapy.

3.4. Detection of Biofilm Formation

The results showed that the capacity of some *P. mirabilis* isolates to form biofilm was detected by phenotypic method which included Congo Red Agar Method (CRA), out of 60 (100%) isolates of *P. mirabilis* only 45 (75%) were give positive results with biofilm formation and appear black dry crystalline colonies on the CRA plates and 15 (25%) were non - biofilm producers when the colonies of *P. mirabilis* producer remained pink or red colored Figure 2.

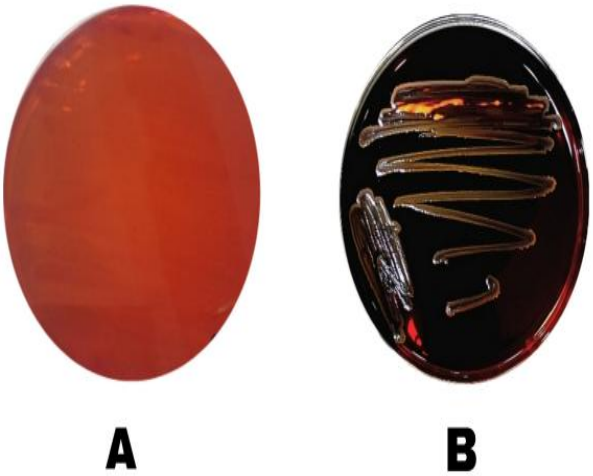

Figure 2: Biofilm formation by some *P. mirabilis* isolates (A) without Biofilm formation and (B) Biofilm formation

According to our results, the results nearly to result conducted by [12] the percentage were (92.05%) of the isolates were biofilm producers, whereas (7.95%) of the isolates were non-producers. *P. mirabilis* pathogenicity is not solely due to the expression of its virulence genes, biofilm formation also adds to the infection's complexity. It is understood that persistent and harmful infections and inflammatory processes are ultimately brought on by biofilms [13]. A biofilm is a collection of microbial cells that adhere to specific surfaces and nearby cells, and it is protected by an extracellular matrix. Biofilms unintentionally aid bacterial survival by facilitating better environmental adaptation and more efficient nutrient utilization [14].

3.5. Detection of Genes That Responsible For Antibiotic Resistance In *P. Mirabilis*

Only 26 isolated of *P. mirabilis* were submitted to investigate antibacterial resistance genes, The results showed that the *sul* gene was detected in *P. mirabilis* isolates , out of 26 (100%) isolates only 20 (77 %) were have *sul1* gene, The results also showed that the *(GyrA)* gene was detected in *P. mirabilis* which were in 25 (96%) isolates, the results display 5 (19%) isolates were have *Ant(2)-1a* gene , Regarding SHV gene was detected in 8(30%) of *P. mirabilis* isolates ,while the results showed that the rmtB gene 24 (92v%) of *P. mirabilis* isolates, as in Figure 3, Figure 4, Figure 5, Figure 6, Figure 7 and Figure 8 respectively.

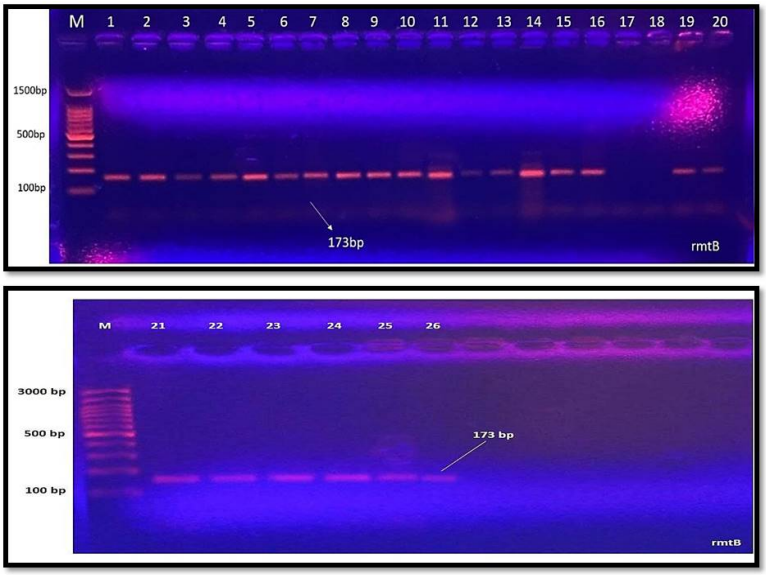

Figure 3: Agarose gel with ethidium bromide stained of mono-plexPCR amplified product from extract DNA of *P. mirabilis* isolates with *(rmtB)* gene primers , performed at (80V, 1.5hr) Lane (L) DNAmolecular size marker (‘100-bp ladder) , Lane (1, 2, 3, 4, 5,6, 7, 8, 9,10, 11,12,13, 14,15,16, 19, 20, 21, 22, 23, 24, 25, 26) show positive results

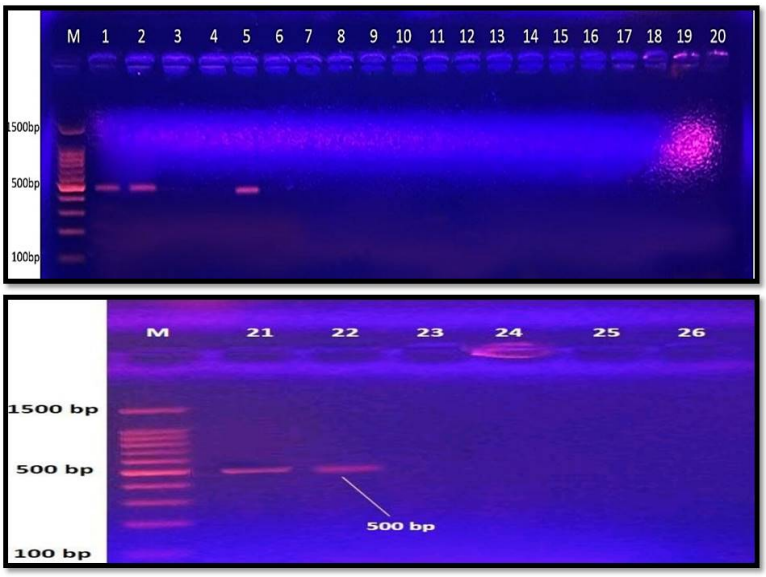

Figure 4: Agarose gel with ethidium bromide stained of mono-plex PCR amplified product from extract DNA of *P. mirabilis* isolates with *(Ant(2)-1a)* gene primers , performed at (80V , 1.5hr) Lane (L) DNA molecular size marker (‘100-bp ladder) , Lane (1, 2, 5, 21, 22) show positive results

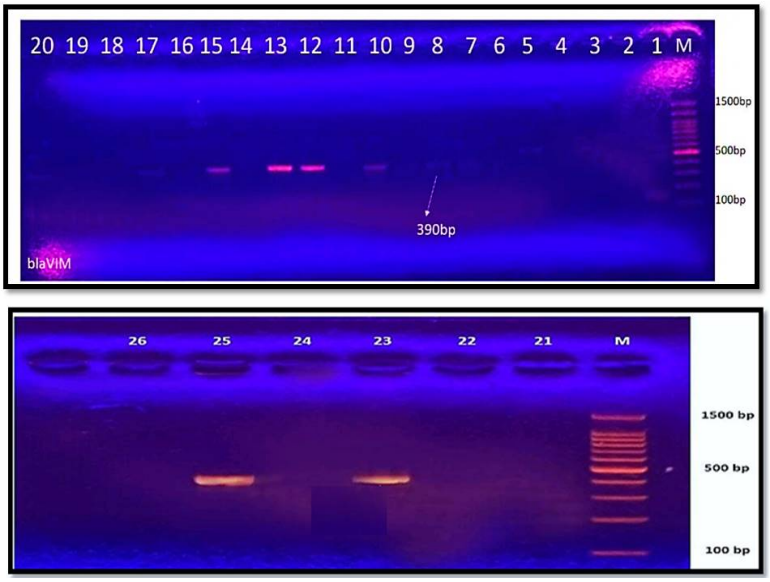

Figure 5: Agarose gel with ethidium bromide stained of mono-plex PCR amplified product from extract DNA of *P. mirabilis* isolates with *(blaVIM)* gene primers, performed at (80V, 1.5hr) Lane (L) DNA molecular size marker (‘100-bp ladder), Lane (7, 8, 10, 12, 13, 15, 17, 20, 23, 25) show positive results

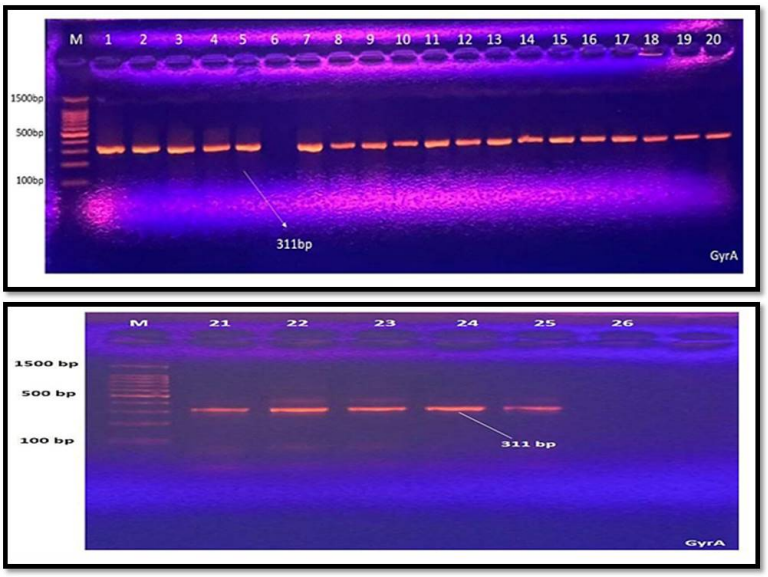

Figure 6: Agarose gel with ethidium bromide stained of mono-plex PCR amplified product from extract DNA of *P. mirabilis* isolates with *(gyrA)* gene primers, performed at (80V, 1.5hr) Lane (L) DNA molecular size marker (‘100-bp ladder), Lane (1, 2, 3, 4, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26) show positive results

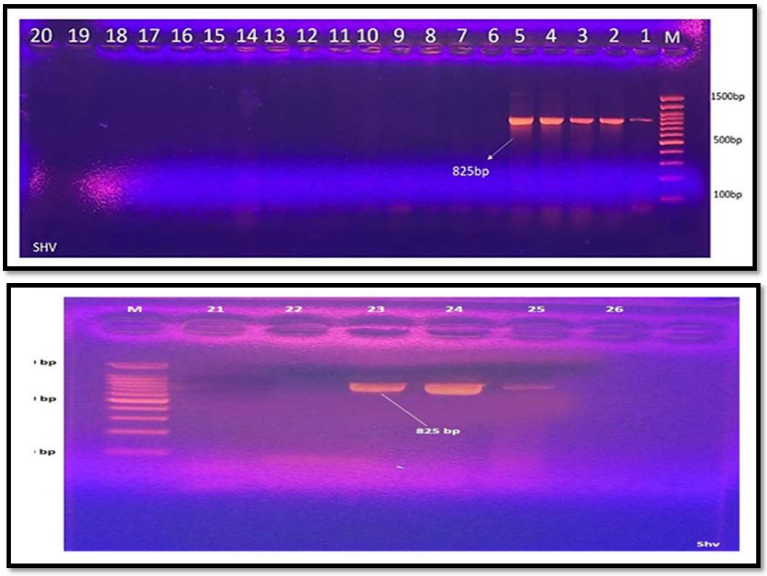

Figure 7: Agarose gel with ethidium bromide stained of mono-plex PCR amplified product from extract DNA of *P. mirabilis* isolates with *(blaSHV)* gene primers, performed at (80V, 1.5hr) Lane (L) DNA molecular size marker (‘100-bp ladder), Lane (1, 2, 3, 4, 5, 23, 24, 25) show positive results

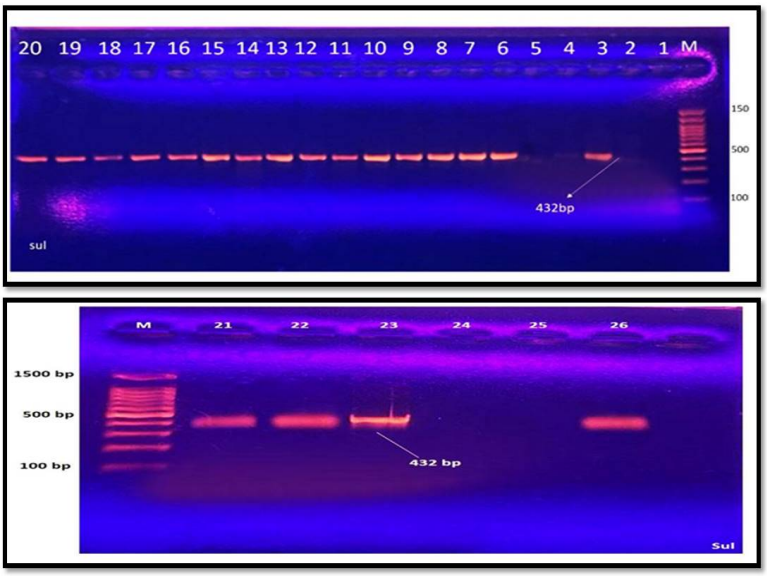

Figure 8: Agarose gel with ethidium bromide stained of mono-plex PCR amplified product from extract DNA of *P. mirabilis* isolates with *(Sul1)* gene primers, performed at (80V, 1.5hr) Lane (L) DNA molecular size marker (‘100-bp ladder) , Lane (3, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 26) show positive results

Table 5: Distribution of resistance genes for *P. mirabilis* isolates.

NO.Of Name Gene	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
sul1	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
blaSHV	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
Ant(2)”-1a	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
gyrA	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
blaVIM	-	-	-	-	-	-	+	+	-	+	-	+	+	-	+	-	+	-	-	-	+	-	-	-+	+	-
rmtB	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+

Antibiotic treatment against infections caused by isolates harboring plasmid mediated or chromosomal quinolone resistance genes is more complicated because of their high potential for transmitting antibiotic resistance among different bacterial species, Sulfonamides are structural analogues of para-aminobenzoic acid (PABA) and competitively inhibit dihydropteroate synthetase,an enzyme that facihites PABA as substrate for the synthesis of dihydrofolic acid (folic acid), Combination Of trimethoprim and sulfamethoxazole with the trade name of cotrimoxazole is the first antibiotic that has been used for the treatment of urinary tract infections, Dihydropteroate synthetase with low affinity for sulfonamides is encoded on a plasmid, which has high speed transfer potential to other organisms. Furthermore, *(SuL1)* gene was known plasmid encoded sulfonamide resistance genes, which produce dihydropteroate synthetase (DHPs) and induce resistance against sulfonamides [15, 16].

Based on our results the *(suLl)* gene has the highest prevalence in *P. mirabilis* strains resistant to Cotrimoxazole existence of *(suL1)* genes in different kinds of clinical isolates, the *(suLl)* gene is mainly associated with class I integrons in many sulfonamide resistant bacteria class 1 integrons play an important role in antibiotic resistance dissemination in many Multidrug Resistant (MDR) Gram-negative bacteria, Acquisition and dissemination of these genes located within the integron structure results in an increase in antimicrobial resistance, According to our results the *(suL1)* gene has the highest prevalence in *P. mirabilis* strains and this is consistent with [17, 18].

Other study results obtained by [19] showed that all *P. mirabilis* isolates (100%) were carried *(suL 1)* gene , also [20] they reported that the frequency of *(suL1)* gene was 94.34 % of the *P. mirabilis* isolates from community and hospital care settings.

The *rmtB* one of genes which are responsible for the resistance in aminoglycoside antibiotics which block protein synthesis by targeting the A site or recognition site located in the 16S rRNA of the bacterial 30S ribosomal subunit where codon— anticodon accuracy is assessed leading to misreading of the genetic code and inhibition of translocation. Bacteria have been several resistance mechanisms to cope with aminoglycosides and the most common being chemically modifying aminoglycoside- modifying enzymes (AMES) [21]. Other studied recorded by [22] they showed that from 30 isolates of *P. mirabilis* 5 (16.7%) isolates were carried *(rmtB)* gene.

Beta-lactam antibiotics are it one of the greatest usually prescribed treatment classes with many therapeutic indications, these advent initial from the 30s of the twentieth century drastically different the scenario of the fight in contradiction of bacterial infectious diseases, the mechanism of action for this antibiotics comprise, the peptidoglycan or murine is a vital basic of the bacterial cell wall this gives mechanical constancy for it, that is an very conserved constituent of within the gram-positive and gram-negative covers, the beta-lactam antibiotics prevent the latter stage in peptidoglycan creation via acyl ting the trans peptidase involved in cross-linking peptides to make peptidoglycan, The goals for activities of beta-lactam antibiotics it called as penicillin-binding proteins (PBPs), the binding, in turn, interrupts the terminal transpeptidation method and gives failure of viability and lysis and by autolytic methods with the bacterial cell, β-Lactamases are by far the greatest significant resistant process in Gram-negative bacilli, with the popularization of genetic techniques, an increasing number of this enzymes have been categorized different in amino acid series and hydrolytic activity for β-lactam antibiotics [23, 24].

Gram-negative bacteria, inducible appearance of β-lactamases is ordinarily originate in chromosomal β-lactamases whereas plasmid-mediated enzymes are usually constitutively expressed improvement appearance for this hydrolytic action is frequently controlled within promoters appear in upstream genes [25].

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