BioGecko Vol 12 Issue 01 2023

ISSN NO: 2230-5807

Detection of MDR *Proteus mirabilis* Genes Isolates From Urinary Tract Infection

Baneen Abdul-hussein and Baydaa A. Hassan
University of Kufa-Faculty of Science-Department of Biology
Corresponding author email: baneenalbayati98@gmail.com

Abstract---This study was completed in laboratories of Biology Department in Faculty of Science. It explains of antibiotics resistance *Proteus mirabilis* that isolated from urinary tract Infection infection patients in the province of Najaf. A total number of samples 250(100%) ,158(63.2%) samples were female , 64(25.6%) samples were male and 28(11.2%) samples were children, so from total specimens of158(63.2%) samples were female, 64(25.6%) samples were male and 28(11.2%) samples were chilran, so from total specimens of 233(93.2%) showed significant bacterial growth 153(65.7 %) of female specimens, 56(24%) of male specimens and 24(10.3%) from children specimens, the results showed that they have been Selected 8bacterial species ,the most common pathogen was *Escherichia coli*82(35.2%) , *Klepsiella pneumoniae* 57(24.5 %), *Proteus mirabilis* 40(17.2%) *S.aureus* 22(9.4%),*P.aeruginosa* 12 (5.2%) ,*Staphylococcu saprophyticus* 10(4.3%), *P.vulgaris* 7(3%) and *Staphylococcu aglacthae*3(1.3%), Antibacterial susceptibility test was conducted for 40 *P.mirabilis* isolates against 20 commonly used antibacterial agents by using the disk diffusion method , the highest rate of resistance was seen withAmpicillin 40/40(100%) followedAmoxicillin- clavulanic Acid 39/40 (97.5%)and Doxycycline 36/40 (90 %) and the low rate resistance was seen withMeropenem 0/40 (0%)and Piperacilline/Tazobactamwas 3/40(7.5%),the results showed that the capacity of some *P.mirabilis* isolates to biofilm formation , from the 40 (100%) isolates of *P.mirabilis*9(22.5%) were biofilm producers high ,7(17.5%) were biofilm producers moderate, 6(15%)were biofilm producers weak and 18 (45%) were non - biofilm producers , the results showed that the(sul 1) gene was detected in *P.mirabilis* isolates, from the 30 (100%) isolates 14 (46.7%) were have Sul1 gene, Regarding rmtB gene the result 30 (100%) isolates of *P.mirabilis* 12(40%) were have rmtB gene, While the results showed that the bla imp was detected in *P.mirabilis* isolates, from the 30 (100%) isolates 5(16%) were have bala imp gene.

Keywords---Proteus mirabilis, infection, resistance gens, urinary tract.

1.Introduction

Urinary tract infections (UTIs) are inflammatory disorders caused by microorganisms that have proliferated abnormally in the urinary system (1). UTIs are known to induce short-term morbidities such as fever, dysuria, lower abdominal pain, and may result in permanent kidney scarring , urinary tract infection is more common in women than in men because of the anatomical proximity of the urethra to gut opening (2). Infections of the urinary tract produced by gram-positive bacteria are thought to be less common than infections caused by gram-negative bacteria, which are often caused by *Enterococcus* species, *S.saprophyticus*, and group B *Streptococcus*(3). 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 , 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 , The development of specific mechanisms of resistance had provoked their therapeutic use, several Enterobacteriaceae strains have been isolated which are resistant to antibiotics , gram-negative bacteria are intrinsically resistant to several antibiotic classes because of the presence of a second , OM compared to gram-positive bacteria which these antibiotics cannot penetrate (4).

2. Materials and Methods

2.1. Samples collection and bacterial identification

A total number of250(urine samples) were collected from patients with Urinary tract infection admitted to AL-Zhraa Hospital and AL-Hakim General Hospital in AL-Najaf Governorate, during the period from(September, 2022 to November, 2022). All samples were collected in a way to avoid any potential contamination, Swabs were taken and close it until transported to advanced Microbiology laboratory/ College of Science / University of Kufa and culturing on different media for 24 hours at cultivate 37 °C 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.mirabilis*isolates 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 (5).

2.3. Detection of biofilm formation for *P.mirabilis* isolates

Biofilm production by isolated from different infection pathogens in our study was detected bymicrotiter plate method (6).

2.4. Molecular Techniques

2.4.1. Extraction of Genomic DNA

Genomic DNA was extracted by using a method of (7).

2.4.2. 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 (2-1) .

Table (2-1): Primers used in this study

Primer Type	Primer sequence (5'-3')	Amplicon size(bp)	Reference
sul1	F: CGGCGTGGGCTACCTGAACGR:GCCGATCGCGTGAAGTTCCG	432	(8)
RmtB	F: GCT TTC TGC GGG CGA TGT AA R: ATG CAA TGC CGC GCT CGT AT	173	(9)
BlaIMP	F: GGAATAGAGTGGCTTAAYTCTC R: GGTTTAAYAAAACAACCACC	232	(10)

2.4.3. 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 (2-2).

Table (2-2): Amplification conditions of genes were used by PCR reactions

Gene Name	Temperature (◦c) / Time					Cycles Number
	Initial Denaturation	Cycling conditions			Final Extension	
		Denaturation	Annealing	Extention		
sul1	94/1 min	98/30 sec	55/30 sec	72/30 sec	72/10 min	30
blalMP	95/3 min	95/30 sec	56/30 sec	72/45 sec	72/5 min	30
rmtB	94/4 min	94/1 min	50 /1 min	72/1.5min	72/5 min	35

3. Results and Discussion

The patients samples of urinary tract infection were obtained from the hospitals in Al-Najaf province , a total of samples 250 (100%) ,158(63.2%) samples were female, 64 (25.6%) were male and 28(11.2%) samples were chidren , so from total specimens of 233(93.2%) showed significant bacterial growth 153(65.7%) of female specimens, 56(24.0%) from male specimens and 24 (10.3%) from children specimenstable (3-1) .

Table (3-1) : Percentage of urinary tract infection samples in Najaf governorate

No	Gender	Number of Sample	Percentage %	Number of Sample that bacterial growth appeared	Percentage%
1	Female	158	63.2 %	153	**65.7 %**
2	Male	64	25.6 %	56	**24 %**
3	children	28	11.2 %	24	**10.3%**
Total		250	100	233	**100%**

Urinary tract infections (UTIs)are the most prevalent bacterial infections that often affect all components of the urinary system , it is the third most common infection after respiratory and gastrointestinal infections and causes a significant morbidity and considerable mortality that affects about 150 million people each year worldwide (11). It is more common in women than in men because of the anatomical proximity of the urethra to gut opening (12) .

3-2: Identification of bacterial species that causes of urinary tract

Many bacterial species are considered to cause urinary tract**.**Bacterial isolates were diagnosed by using the bacterial cultured . The biochemical tests and Vitek 2 system for the bacterial isolates were investigated in the laboratory, they have been elected 8 bacterial species *E.coli* , *Klebsiella pneumoniae* , *Proteus mirabilis* , *Staphylococcu aureus* ,*Psedomonas aeruginosa*, *Staphylococcu saprophyticus* ,*Proteus vulgaris and Streptococcus aglacthae*, the most common pathogen was *Escherichia coli*82(35.2%) , *Klepsiella pneumoniae*57(24.5%), *Proteus mirabilis*40(17.2%) *S.aureus*22(9.4 %),*P.aeruginosa* 12(5.2%) ,*S.saprophyticus*10(4.3%) ,*P.vulgaris* 7(3%) ,*S.aglacthae* 3(1.3%) Figure (3-1) .

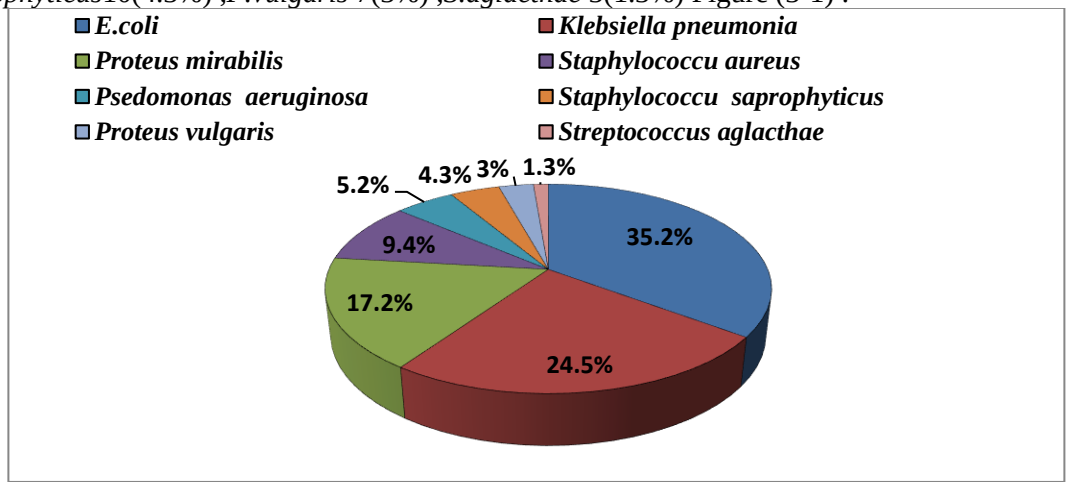


Figure (3-1): Percentage of frequency of bacterial species that isolated from urinary tract infection samples

3.3. Antibiotics susceptibility of *P.mrabilis* isolates

Antimicrobial susceptibility test towards twenty antibiotics was determined using agar disc diffusion test (Kirby-Bauer method) according to the Clinical Laboratory Standards Institute (CLSI) guidelines (2022).in this study ,revealed that (40) isolates of *P.mirabilis*showed a variable levels of resistance to antibiotics, as shown in the table(4-3) .

Table (3-2) : Antibiotic susceptibility test for 40 *P.mirabilis*isolates

Type of antibioticclass (subclass)	Antibiotic disk	No. (%) antibiotic Resistance	
		Resistance	Susceptible
Fluoroquinolones	Ciprofloxacin	28 (70%)	12(30%)
	Norfloxacin	6(15 %)	34 (85%)
Quinolones	Nalidixic Acid	19 (47.5%)	21 (52.5%)
Nitrofurans	Nitrofurantoin	33 (82.5%)	7 (17.5%)
β-lactams cephalosporines	Ceftazidime	29 (72.5%)	11 (27.5%)
	Ceftriaxone	28 (70%)	12 (30%)
	Cefixime	21 (52.5%)	19 (47.7%)
	Cephalothin	31(77.5%)	9(22.5%)
β-lactams carbapenems	Meropenem	0 (0%)	40(100%)
	Impenem	2 (5 %)	38 (95%)
β-lactams penicillns	Amoxicillin- clavulanic acid	39 (97.5%)	1 (2.5%)
Aminoglycosides	Tobramycin	29(72.5 %)	11 (27.5%)
	Gentamicin	8 (20%)	32 (80 %)
	Amikacin	7 (17.5%)	33 (82.5%)
Sulfonamides (antifolate)	Trimethoprime-sulfamethaxazol	34 (85%)	6 (15%)
Tetracyclins	Doxycycline	36 (90%)	4 (10%)
	Tetracycline	32 (80%)	8 (20%)
Polymyxin	Colistin	37 (92.5%)	3 (7.5%)
Penicillins	Piperacillin /Tazobactam	3 (7.5%)	37 (92.5%)
	Ampicillin	40 (100%)	0 (0 %)

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 (13). Inadequate surveillance systems and dependence on reliable microbiological techniques also contribute to the improper prescription of antibiotics (14).

According to (15), *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.

Highly resistance was shown against ampicillin with (95%) resistance another study(16) recorded (75%), while other researcher (17) recorded (97.37 %) and (18) who demonstrated that (79%) of isolates were resistant to ampicillin. The problem in treating patients caused by the rise in antibiotic resistance raises the mortality rate. Meropenem, impenem, amikacin,gentamicin , norfloxacin, and Piperacillin /Tazobactam had the lowest rates of resistance discovered in this study, this may be attributable to the poor usage of these antibiotics in Al-Najaf hospitals.

Meropenem was in the first place it was the most effective and sensitive drug against *P.mirabilis* isolates in the current study, This result agreed with (19) who found the sensitivity to this drug 100%, And also resemble to (20), who documented that the resistance of *P. mirabilis* was 4.5%, while this result disagree with (21) observed that Proteus mirabilis bacteria had a 47.1% resistance rate to this antibiotic, Further more (22) indicated that the resistance of all of its isolates to meropenem was 0%.

The occurrence of resistance within *P.mirabilis* to several antibiotics is an emerging problem, extremely complex and made UTIs management progressively more costly and challenging. Which limited the choices for selecting the appropriate drug for the treatment of *P.mirabilis* the high incidence of *P.mirabilis* infections, has been known widely for its contribution to the worldwide dissemination of multidrug resistance (MDR). When a bacterial strain resistant to at least three classes of antibiotics defined as MDR bacteria (23). In the present study demonstrated that all *P. mirabilis* isolates (100%) were MDR showing resistance to a minimum of three classes of the antibiotics.

3.4:Detection of Biofilm Formation

The results showed that the capacity of some *P.mirabilis* isolates to biofilm formation , from the 40 (100%) isolates of *P.mirabilis* 9(22.5%) were biofilm producers high ,7(17.5%) were biofilm producers moderate, 6(15%)were biofilm producers weak and 18 (45%) were non - biofilm producers Figure (3-2) .

According to our results, the results similar to result conducted by (19) the percentage were 9(15%) strong, 8(13.33%) moderate, 10(16.66%) weak, and 33(55%) was non-producer to biofilm.

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 (24). 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 (25) .

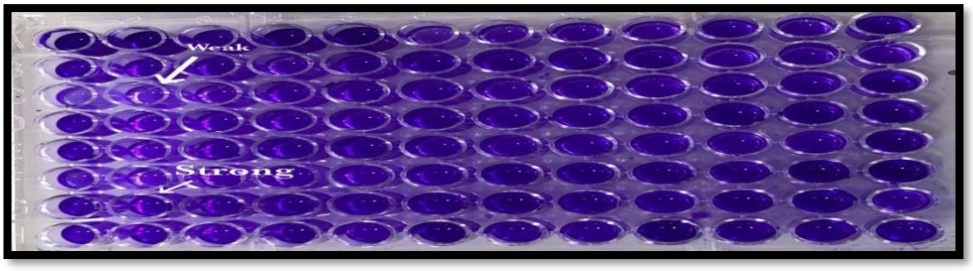


Figure (3-2): Biofilm formationof *P.mirabilis*

3.5: Detection of genes that responsible for antibiotic resistance in *P. mirabilis*

The results showed that the *Sul1*gene was detected in isolates, from the 30 (100%) isolates 14 (46.7%) were have *Sul1* gene Figure (3-1) , The results also showed that the *rmtB* gene was detected in *P. mirabilis* isolates from the 30 (100%) isolates 12 (40%) were have *rmtB*gene Figure (3-2). The results also displayed the *bla imp*was detected in *P.mirabilis* isolates , from the 30 (100%) isolates 5(16%) were have *bla imp*gene Figure (3-3) .

Sulfonamides are structural analogues of paraamino benzoic acid (PABA), which competitively inhibit dihydropteroate synthetase activity, Simultaneous prescription Of dihydrofolate reductase (trimethoprim) with sulfonamides creates a synergistic antimicrobial activity in bacterial infections, 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 (26). Dihydropteroate synthetase with low affinity for sulfonamides is encoded on a plasmid, which has highspeed 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 (27). The (suLl) gene is part Of 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 (28) . According to our results the *(sul1)* gene has the highest prevalence in *P.mirabilis*strains and this is consistent with (29).

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) (30).

Beta-lactam antibiotics are one of the most commonly drug classes with several clinical indications, the mechanism of action for this antibiotics include, the peptidoglycan or murein is a vital constituent of the bacterial cell wall that gives mechanical stability to it, it is conserved constituent of both the gram-positive and gram-negative envelopes. The beta-lactam antibiotics inhibit the last step in peptidoglycan synthesis by acylating the transpeptidase involved in cross-linking peptides to form peptidoglycan (31).The goals for activities of beta-lactam antibiotics it named as penicillin-binding proteins (PBPs), the binding, in turn, interrupts the terminal transpeptidation way and provides failure of viability and lysis, and by autolytic methods with the bacterial cell, β-Lactamases are by far the greatest resistant prosess in Gram-negative bacilli, with the popularization of genetic methods, an increasing number of this enzymes have been categorized changed in amino acid series and hydrolytic activity for β-lactam antibiotics (32).IMP-producing *P. mirabilis* have been identified in only two studies from the United States (33) .

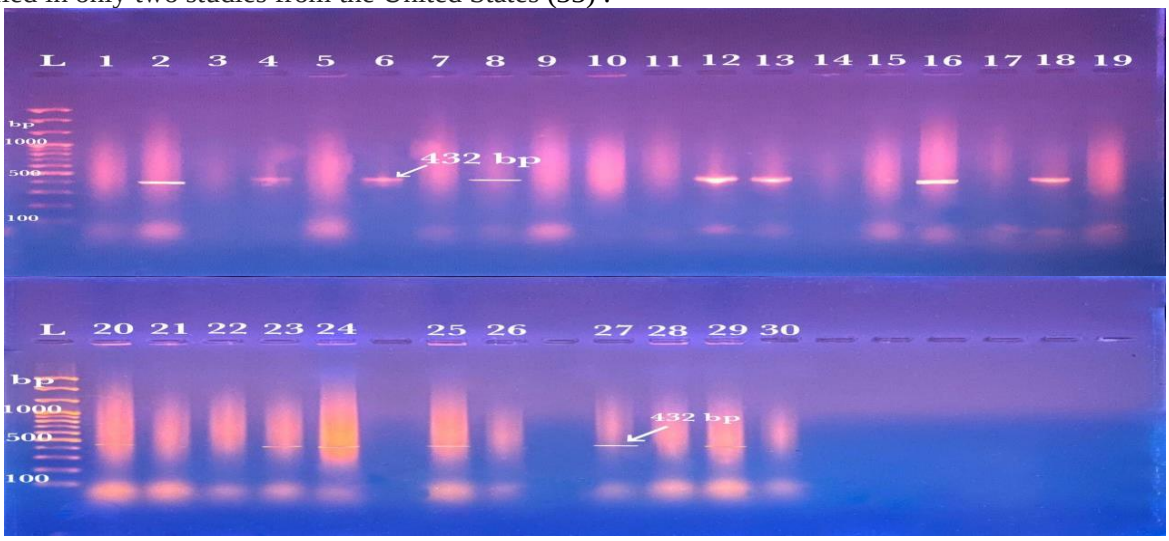
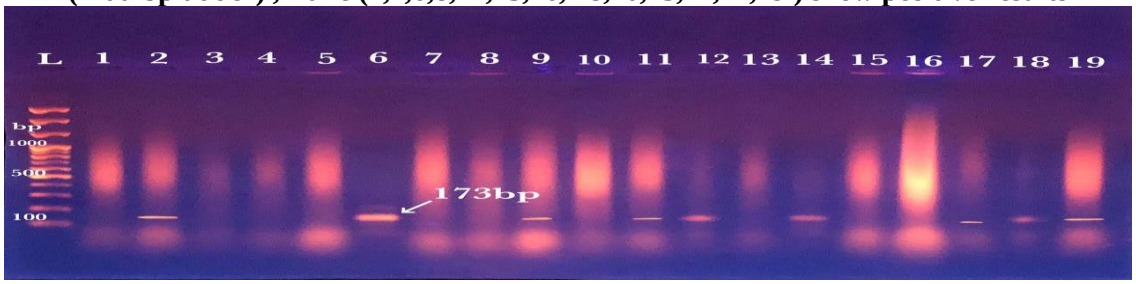


Figure (3-2) Agarose gel with ethidium bromide stained of mono-plex PCR amplified product from extract DNA of *P. mirabilis* isolates with (*Sul1*) gene primers , Lane (L) DNA molecular size marker (`100-bpladder) , Lane (2,4,6,8,12,13,16, 18,20,23,24,27,29) show positive results



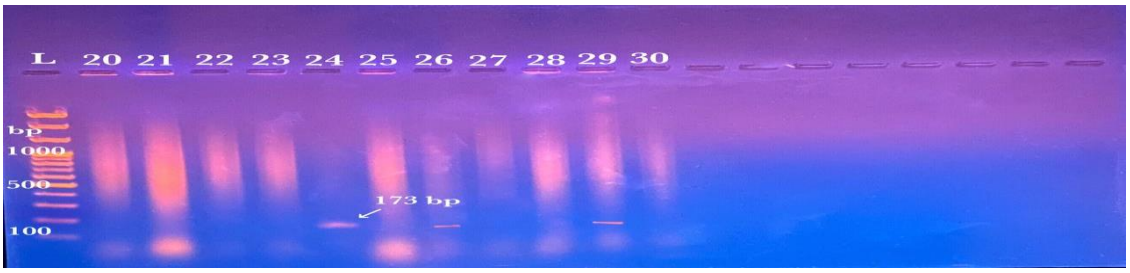


Figure (3-3) Agarose gel with ethidium bromide stained of mono-plex PCR amplified product from extract DNA of P. mirabilis isolates with (rmtB) gene primers , Lane (L) DNA molecular size marker (100-bp ladder) , Lane (2,6,9,11,12,14,17,18,19,24,26,29) show positive results

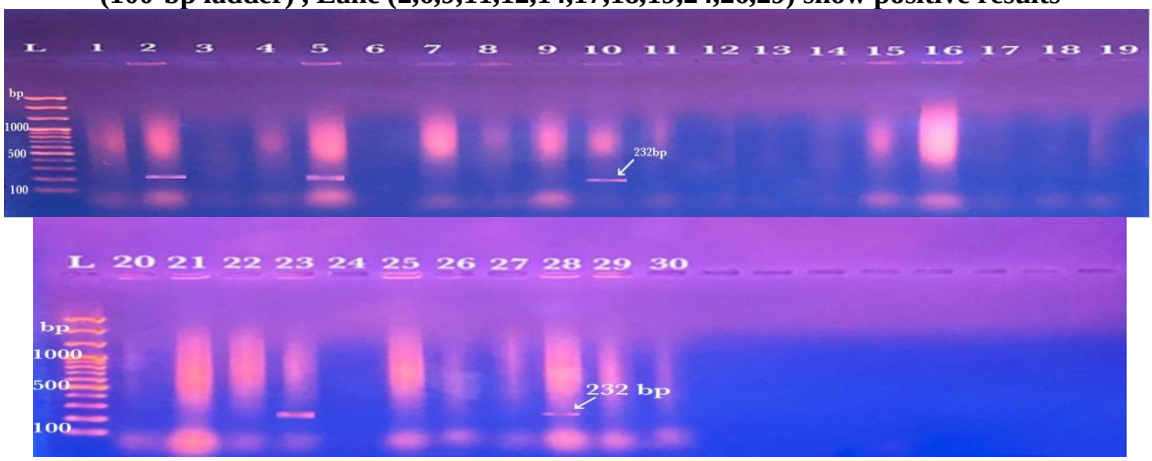


Figure (3-4) Agarose gel with ethidium bromide stained of mono-plex PCR amplified product from extract DNA of P. mirabilis isolates with (bla IMP) gene primers , Lane (L) DNA molecular size marker (100-bp ladder) , Lane (2,5,10,23,28) show positive results

References

1. Malik , S., Rana, J. S., and Nehra, K. (2021). Prevalence and antibiotic susceptibility pattern of uropathogenic *Escherichia coli* strains in Sonipat region of Haryana in India. *Biomed. Biotechnol. Res. J.* 5, 80–87. Malik,
2. S., Rana, J. S., and Nehra, K. (2021). Prevalence and antibiotic susceptibility pattern of uropathogenic *Escherichia coli* strains in Sonipat region of Haryana in India. *Biomed. Biotechnol. Res. J.* 5, 80–87.
3. Teo, chee loong. (2022). Antimicrobial Study Of Pyroligneous Extract From Rhizophora Apiculate Against Urinary Tract Pathogens. Jurnal Teknologi, 1, 49–55.
4. Rahman , AE., Iqbal ,A., Hoque DE, et al. (2017) . Managing neonatal and early childhood syndromic sepsis in sub-district hospitals in resource poor settings: improvement in quality of care through introduction of a package of interventions in rural Bangladesh. PloS one. , 12:10.1371.
5. Clinical and Laboratory Standards Institute (CLSI) .(2022).Performance Standards for Antimicrobial Susceptibility Testing; 29thed. supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute.

6. Tahmourespour, A. and Kermanshahi, R.K. (2011) 'The effect of a probiotic strain (Lactobacillus acidophilus) on the plaque formation of oral Streptococci', Bosnian journal of basic medical sciences, 11(1), p. 37.
7. Yang, J.L.; Wang, M.S. ; Cheng, A.C.; Pan, K.C. ; Li, C.F. and Deng, S.X. (2008). A simple and rapid method for extracting bacterial DNA from intestinal microflora for ERIC-PCR detection. World J. Gastroenterol., 14: 2872-2876.
8. Minh, C. ;Phuc, N.; Paul-Louis, W.; Mathilde, B.; Antoine, A.; Roland, L. and Annie, C. (2009). Escherichia coli as Reservoir for Macrolide Resistance Genes. Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 15, No. 10,
9. Kashfi M, Hashemi A, Eslami G, et al. The Prevalence of Aminoglycosidemodifying Enzyme Genes among Pseudomonas aeruginosa Strains Isolated From Burn Patients. Arch. Clin. Infect Dis. 12(1), e40896 (2017) .
10. Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis. 2011;70(1):119– 123. doi:10.1016/j.diagmicrobio.2010.12.002 .
11. Saha, M. H., Mishra, S. K., Dash, S., Mishra, A., & Satpathy, J. (2020). In-vitro study of the activity of some medicinal plant leaf extracts on urinary tract infection causing bacterial pathogens isolated from indigenous people of Bolangir district, Odisha, India. BioRxiv. https://doi.org/10.1101/2020.06.25.172650
12. Fazly Bazzaz, B. S., Fork, S. D., Ahmadi, R., and Khameneh, B. (2021). Deep insights into urinary tract infections and effective natural remedies. *Afr. J. Urol.* 27, 1–13.
13. Konate, A., He, X., Zhang, Z., Ma, Y., et al. (2017) 'Magnetic (Fe3O4) nanoparticles reduce heavy metals uptake and mitigate their toxicity in wheat seedling', Sustainability, 9(5), p. 790
14. Wellington, E.M.H., Boxall, A.B.A., Cross, P., Feil, E.J., et al. (2013) 'The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria', The Lancet infectious diseases, 13(2), pp. 155– 165.
15. Al-Jumaily, E. and Zgaer, S.H. (2016) 'Multidrug resistant Proteus mirabilis isolated from urinary tract infection from different hospitals in Baghdad City', Int. J. Curr. Microbiol. App. Sci, 5(9), pp. 390–399.
16. Attallah, N.A. and Farhan, M.B. (2020) 'Bacteriological Study and Investigation of Some Virulence Factors of Proteus mirabilis Bacteria Isolated from Urinary Tract Infection Patients in Ramadi City.', Indian Journal of Forensic Medicine & Toxicology, 14(4).
17. Shabeeb, B.T., Alghanimi, Y.K. and Ahmed, M.M. (2018) 'Molecular and Bacteriologic Study of â-lactam Resistance Proteus mirabilis associated with Urinary Tract Infection in Holy Karbala province, Iraq', Journal of Pharmaceutical Sciences and Research, 10(3), pp. 549–555.
18. El-Gamasy, M.A. (2017) 'Prevalence of infective organisms of infections of urinary tract in a sample of Arab infants and children', Journal of Integrative Nephrology and Andrology, 4(4), p. 136.
19. Alkhalidy, R.Muayad. and Rasmiya, A.Aburesha. (2021) Detection of the presence of luxS and rsbA genes and their relation with biofilm mediating antibiotic resistance in *Proteus mirabilis*. Master Thesis. Baghdad University.
20. Mirzaei, A., Habibi, M., Bouzari, S. and Karam, M.R.A. (2019) 'Characterization of antibiotic-susceptibility patterns, virulence factor profiles and clonal relatedness in Proteus mirabilis isolates from patients with urinary tract infection in Iran', Infection and drug resistance, 12, p. 3967.
21. Kadhim, A.S. (2017) 'Antimicrobial resistance patterns and extended spectrum beta-lactamases producing by Proteus mirabilis isolated from different sources', Al-Mustansiriyah Journal of Science, 28(1), pp. 47–54.

22. Zhang, Y., Zhuang, Y., Geng, J., Ren, H., et al. (2016) 'Reduction of antibiotic resistance genes in municipal wastewater effluent by advanced oxidation processes', Science of the Total Environment, 550, pp. 184–191.
23. Shaik, S., Ranjan, A., Tiwari, S.K., Hussain, A., et al. (2017) 'Comparative genomic analysis of globally dominant ST131 clone with other epidemiologically successful extraintestinal pathogenic Escherichia coli (ExPEC) lineages', MBio, 8(5), pp. e01596-17.
24. Fusco, A., Coretti, L., Savio, V., Buommino, E., et al. (2017) 'Biofilm formation and immunomodulatory activity of Proteus mirabilis clinically isolated strains', International journal of molecular sciences, 18(2), p. 414.
25. Kwiecinska-Pirog, J., Skowron, K., Bartczak, W. and GospodarekKomkowska, E. (2016) 'The ciprofloxacin impact on biofilm formation by PProteus mirabilis and P. vulgaris strains', Jundishapur journal of microbiology, 9(4).
26. Suzuki S, Hoa VT. (2012). Distribution of quinolones, sulfonamides tetracyclines in aquatic environment and antibiotic resistance in indochina. Front Microbiol., 367.
27. Wu S, Dalsgaard A, Hammerum AM, Porsbo Ll. Jensen LB. (2010) .Prevalence and characterization of plasmids carrying sulfonamide resistance genes among Escherichia coli from pigs, pig carcasses and human. Acta Vet Scand.;52:47.
28. Chuanchuen R, , Koowatananukul C, Khemtong S. Characterization of class 1 integrons with unusual 3' conserved region from Salmonella enterica isolates. Southeast Asian J Ti•op Med Public Health.. 2008 39(3.) ;419_24.
29. Penghao Lv , Guijuan Hao , Yanli Cao, Lulu Cui , Guisheng Wang and Shuhong Sun 1.(2022). Detection of Carbapenem Resistance of Proteus mirabilis Strains Isolated from Foxes, Raccoons and Minks in China. Biology .11(292) .
30. El-Badawy, M.F.,Tawakol, W.M., El-Far, S.W., Maghrabi, I.A., Al-Ghamdi., SA., Mansy,M.S., Ashour, M.S., and Shohayeb, M.M. (2017). Molecular identification of aminoglycoside-modifying enzymes and plasmid-mediated quinolone resistance genes among klepsiella pneumoniae clinical isolates recovered from Egyptian patients. Int. J. Microbiol.: 8050432.
31. Eckburg, P.B., Lister, T., Walpole, S., Keutzer, T., Utley, L., Tomayko, J., Kopp, E., Farinola, N., and Coleman, S. (2019). Tolerability, Pharmacokinetics, and Drug Interaction Potential of SPR741, an Intravenous Potentiator, after Single and Multiple Ascending Doses and When Combined with β-Lactam Antibiotics in Healthy Subjects. Antimicrob Agents Chemother. ;63(9).
32. Bush, K. (2010). Alarming β-lactamase-mediated resistance in multidrugresistant Enterobacteriaceae. Curr. Opin. Microbiol. 13, 558–564.
33. Dixon N., Fowler R. C., Yoshizumi A., Horiyama T., Ishii Y., Harrison L., et al. (2016). IMP-27, a unique metallo-β-lactamase identified in geographically distinct isolates of *Proteus mirabilis*. *Antimicrob. Agents Chemother.* 60 6418–6421. 10.1128/AAC.02945-15.