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Detection of *fla*A, *fli*C , *mrp*A and *rsb*A Gene in *proteus mirabilis* Multidrug Resistance Isolated from Different Clinical Sources in Baquba City

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ABSTRACT

Background: *P. mirabilis* possesses multiple virulence factors that enable them to cause infection in various anatomical sites of the human body, including endotoxin, and some of them are secreted into a medium such as the enzyme that analyzes urea, hemolytic enzyme, protease, biofilm, DNase, and others. Excessive use of antibiotics has led to the emergence of multidrug resistance and multidrug resistance bacteria that cause serious infections that are difficult to treat.

Objectives: The study aims to molecular investigation of the virulence genes in *Proteus mirabilis* multidrug resistant.

Methods: A total of 100 samples collected from different clinical sources (urinary tract infections, wounds, stool) in sterile containers in different hospitals in Baquba city (Baquba Teaching Hospital and Al-Batool Hospital) were subjected to antibiotic susceptibility study using disc-diffusion method. Molecular detection of virulence genes using polymerase chain reaction (PCR) analysis and amplicons sequenced.

Results: The results showed that all isolates had multiple resistance against antibiotic, and the highest resistance was against antibiotic Trimethoprim and lowest resistance towards two antibiotic Imipenem and Amikacin . PCR analysis revealed the presence of *fla*A ,fliC, *mrp*A and *rsbA* were present in all isolates in a ratio 100% .

Conclusion: Isolates of *Proteus mirabilis* bacteria, which have virulence factors, it causes many diseases and susceptible to infection through their ability to move, transfer, adhere, and form a biofilm.

Keywords: *Proteus mirabilis*; urinary tract infection; virulence factor, *rsb*A gene.

INTRODUCTION

In both hospitals and the general populace, urinary tract infections (UTIs) are the most common bacterial diseases effecting, ranging in intensity from an infection without symptom to acute prostatitis, pyelonephritis, cystitis, and urethritis. This is among the most common disorders, impacting individuals of all ages. There's many *Proteus* species, with *Proteus mirabilis* being one of the most important. This pathogen is classified as an opportunistic pathogen that affect immune-compromised patients with a variety of diseases and causes nosocomial infections and urinary tract infections [(1)].

Dependent on the patient's location and type, it is also known as Proteus UTI. All patients with catheters are at risk for lethal infections, including UTI, urolithiasis formation, congestion of the urinary tract, bladder stones, kidney stones, and bacteriuria [(2)]. Protease, an enzyme which helps in the breakdown of protein, and urease, an enzyme which catalyses the hydrolysis of urea, among characteristics of the virulence factors and enzymes which *P. mirabilis* possesses. Toxins such as hemolysin, a hemolytic toxin, and proteus toxin agglutinin are found [(3)].

Additionally, *P. mirabilis* has a secondary virulence factor that participates in adhesions, flagella, and colonization [(4)]. Urinary pathogens' virulence factors increase subsequent infections and adherence to mucosal surfaces. The multiresistant enterobacteria that cause UTI are a significant public health issue. Antibiotic resistance is still a big issue, particularly in underdeveloped nations wherever sanitation is still a concern and antibiotic usage is often indiscriminate and poorly monitored [(3)]. The Enterobacteriaceae with the lowest rates of extended-spectrum beta-lactamases (ESBL) and carbapenemase synthesis along with UTIs is *Proteus spp*. Due to the limited antibiotic penetration and development of resistant genes that result in antibiotic resistance, indwelling medical devices (IMDs) are the most resistant to biofilm-producing microbial invaders [(5)]. Due to the pathogen's capacity to mediate urea hydrolysis via the urease it generates, which generates tissue necrosis and inflammation at the infection site and prevents antibiotics from contacting the pathogen, *P. mirabilis* infections are hard to treat, last an a while, and commonly result in death [(6)].

The frequent use of antibiotics leads to antibiotic resistance and the emergence of antibiotic resistance genes, especially in Gram-negative bacteria [(7)]. This is considered as one of the most serious chronic illnesses. A sensory protein that governs swarming behavior is represented by the *rsb*A gene. RsbA was shown to promote gene expression for extracellular polysaccharide production and biofilm formation, showing it might function as a protein sensor of environmental conditions. A histidine-containing phosphor transmitter is expressed by rsbA gene . Nanoparticles of metal oxides having a size range of 1–100 nm represent a new orientation that is increasingly being progressed for use in research and medically care related implementation [(8)]. Due to their low production costs, safety, and simplicity of preparation, ZnO NPs are of the greatest interest [(9)].

It has several medicinal uses, including the delivery of medications, anti-cancer, anti-diabetic, antibacterial, antifungal, and agronomic qualities [(10)].

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ZnO nanoparticles' antimicrobial properties are poorly understood [11]. The World Health Organization (WHO) has shown that ZnO nanoparticles have no effect on human cells at different concentrations even the high, which was detected by numerous tests and assays. [12]

The study aims to molecular investigation of the virulence genes in *Proteus mirabilis* multidrug resistant.

MATERIALS AND METHODS

The study was approved by the Ethics Board of the university of Diyala and informed written consent was taken from each participant 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.

Isolation and Identification of *proteus mirabili*s

A total of 100 samples were collected from different clinical sources (urinary tract infections, wounds, stool) in sterile containers in different hospitals in Baquba city (Baquba Teaching Hospital and Al-Batool Hospital). During sample collection from 13/10/2020 to 12/3/2021.

The samples were cultured on MacConkey`s agar and blood agar plates, and then incubated for 24 hours at 37◦C. The isolates were identified depending on the morphological and biochemical tests and compared to the scheme described by [13]. Morphological and Microscopic Identification: it was possible to see the form, size, texture, and colony organization of the bacteria. Colonies were isolated and stained using Gram stain. In order to determine their form and length, they were ultimately viewed under a microscope. Testing biochemical reactions using indole and oxidase [14] .

Antimicrobial susceptibility

Seven antimicrobial discs to determine their sensitivity, and the antimicrobial susceptibility test (CLSI, 2020). Bacterial isolates were grown in MacConkey broth over night at 37°C. Muller Hinton agar is made and put in petri dishes. In 5 ml of ordinary saline, isolated colonies were suspended using a vortex. It was examined for turbidity in compared to the standard McFarland solution. Seven antimicrobial discs were used to assess the sensitivity of twenty isolates of *Proteus mirabilis* which was treated by spread cotton swabs in three separate directions by rotating the plate by 60 °C for each direction. For a few minutes at room temperature, the plate was turned upside down at room temperature. The plates were comprised of various antibiotics, which were then incubated at 37°C overnight. With the help of a zone inhibition ruler, inhibition zones were measured in millimeters and the results were compared with those of the National Committee for Clinical Laboratory Standards.

Molecular detection of virulence genes

Genomic DNA was extracted by DNA extraction kit (Promega, USA) and stored at -20 °C. PCR approach was used to detect the presence of virulence genes including (*fla*A, *fli*C, *mrp*A and *rsb*A). The primers of this study were specifically designed and synthesized by NCBI – Genbank using a program Primer 3 plus design. Using agarose gel at a concentration of 2% with a potential difference of 100 volts for 60 minutes. The primer sequences, their annealing temperatures and product sizes are given in Table 1.

Table 1: list of primers which were used in this study

primer sequence (5 -3)	**Target genes**	**Size(bp)**	**Tem** °C	Reference
F/ TTC TTA CTG ATA AGA CAT TG **R/ATT TCA GGA AAC AAA AGA TG**	***mrp* A**	565bp	**95°C One cycle** **95°C 30 cycle** **40°C 30cycle** **72°C 30 cycle** **72°C** One cycle	(16)
F/AGG ATA AAT GGC CAC ATT G **R/CGG CAT TGT TAA TCG CTT TT**	***fla*A**	417bp	**95°C One cycle** **95°C 30 cycle** **54.2°C 30cycle** **72°C 30 cycle** **72°C One cycle**	(17)
F/TTG AAG GAC GCG ATC AGA CC **R/ACT CTG CTG TCC TGT GGG TA**	*rsb* A	467bp	**95°C One cycle** **95°C 30 cycle** **58°C 30cycle** **72°C 30 cycle** **72°C One cycle**	(18)
F/ATG GCA CAA GTC ATT AAT R/ACG TAA CAG AGA CAG AAC A	*Fli* C	1095bp	**95°C One cycle** **95°C 30 cycle** **54°C 30cycle** **72°C 30 cycle** **72°C One cycle**	(19)

Ethical approval:
The study was approved by the Ethics Board of University of Diyala.

RESULTS

Isolation of *P. mirabilis*

This study included 100 samples of different clinical sources (urinary tract infections, wounds, stool), the rate of male patients was 44% and female patients 56% their age ranged between 1 to 40 years. It was twenty-seven isolates of *Proteus spp*. were recovered included 20 isolates of *P. mirabilis* and 7 isolates of *P. vulgaris*.

Antimicrobial Susceptibility

The sensitivity of P. mirabilis strains isolated from (urinary tract infections, wounds, stool) was tested against antibiotics, including Cefotaxime, Amoxicillin-Clavulanic acid, Trimethoprim, Piperacillin, Ciprofloxacin, Imipenem, Amikacin in Table (2).

Table 2: The susceptibly pattern of twenty *Proteus mirabilis* isolates to seven antimicrobial agents

Antibiotic	Resistant	Intermediate	Sensitive
Amoxicillin-Clavulanic acid	(85%)17	(0)0	(15%)3
Cefotaxime	(80%)16	(0)0	(20%)4
Trimethoprim	(80%)16	(0)0	(20%)4
Piperacillin	(30%)6	(0) 0	(70%) 14
Ciprofloxacin	(20%)4	(10%)2	(70%)14
Imipenem	(25%)5	(10%)2	(65%)13
Amikacin	(25%)5	(10%)2	(65%)13

Molecular Detection of Virulence Genes

The results of the present study are show that 20(100%) of Proteus mirabilis isolates give positive result at 467bp, this result was shown in Figure (1).

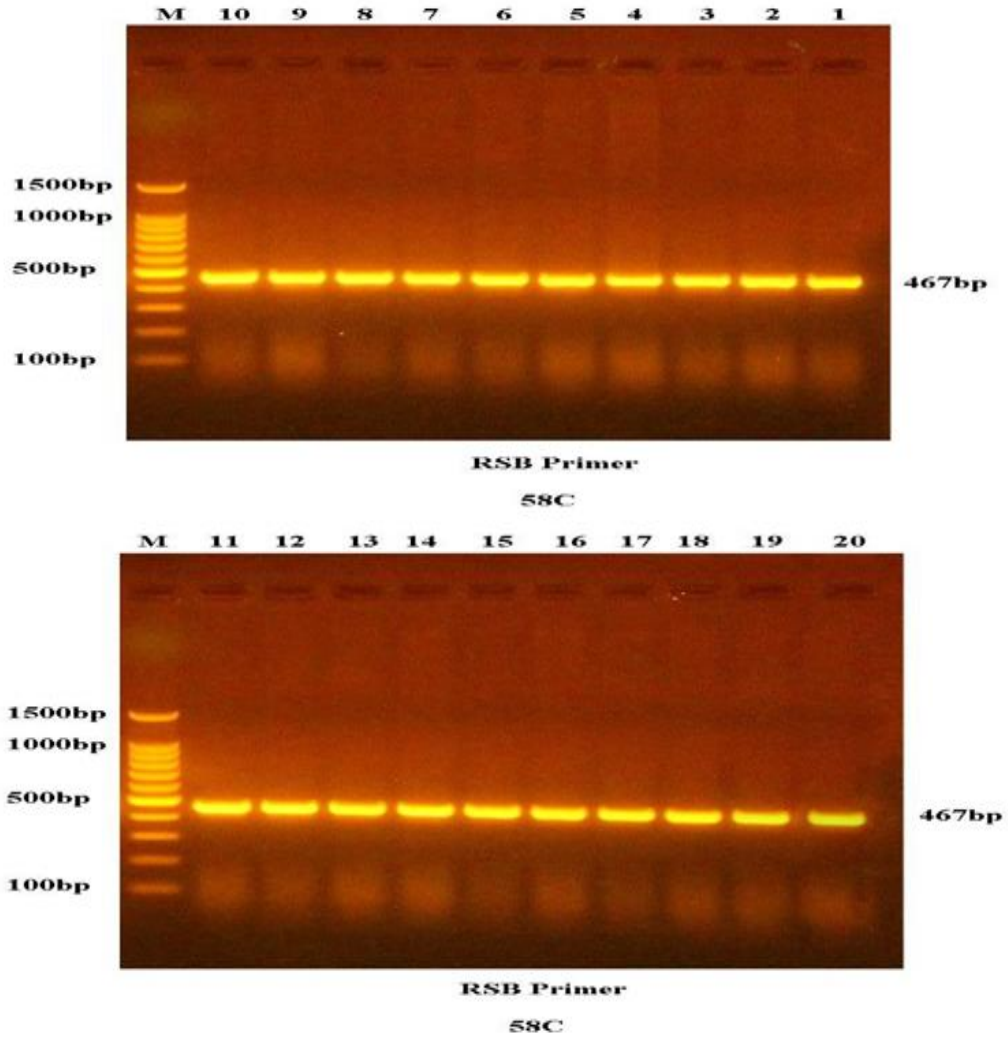

Figure 1. Electrophoresis for the *rsb*A gene where the result showed appearance of the gene in all isolates. M: Ladder with (1500bp) and agarose 2% at a voltage difference of 100 volts for 60 minutes.

The results of PCR amplification to specific *fla* A primers indicated that 20(100%) . The result of electrophoresis showed of the present of a segment of length 417bp belonging to the *flaA* gene, this result was shown in Figure (2) .

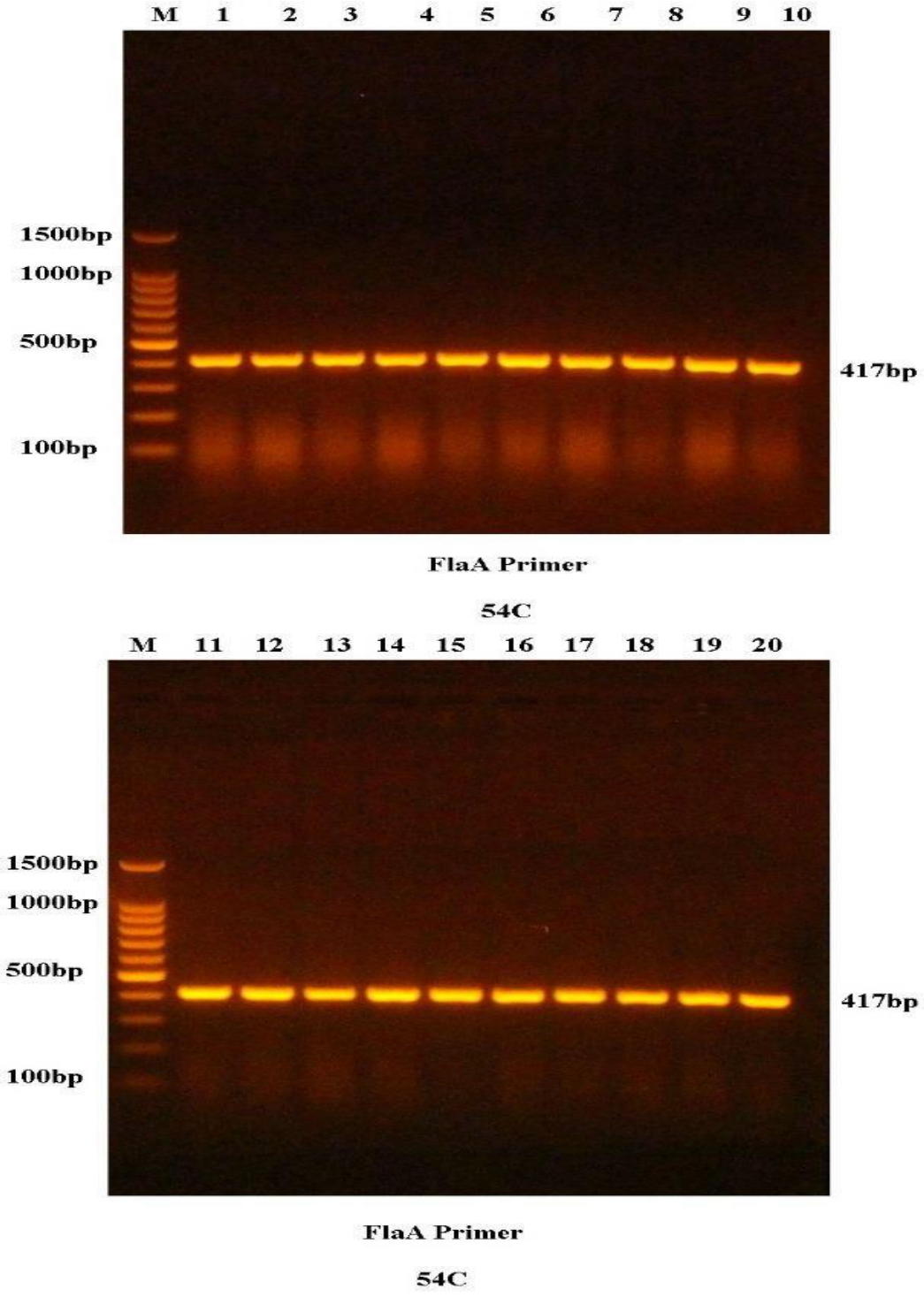

Figure 2. Showed the electrophoresis of the *fla*A gene and the appearance of the gene in all isolates. M: Ladder with (1500bp) and agarose 2% at a voltage difference of 100 volts for 60 minutes.

The result showed that *mrp A* gene was present in all isolates . The result of electrophoresis showed of the present of a segment of length 565bp belonging to the *mrpA* gene . This result was shown in figure (3).

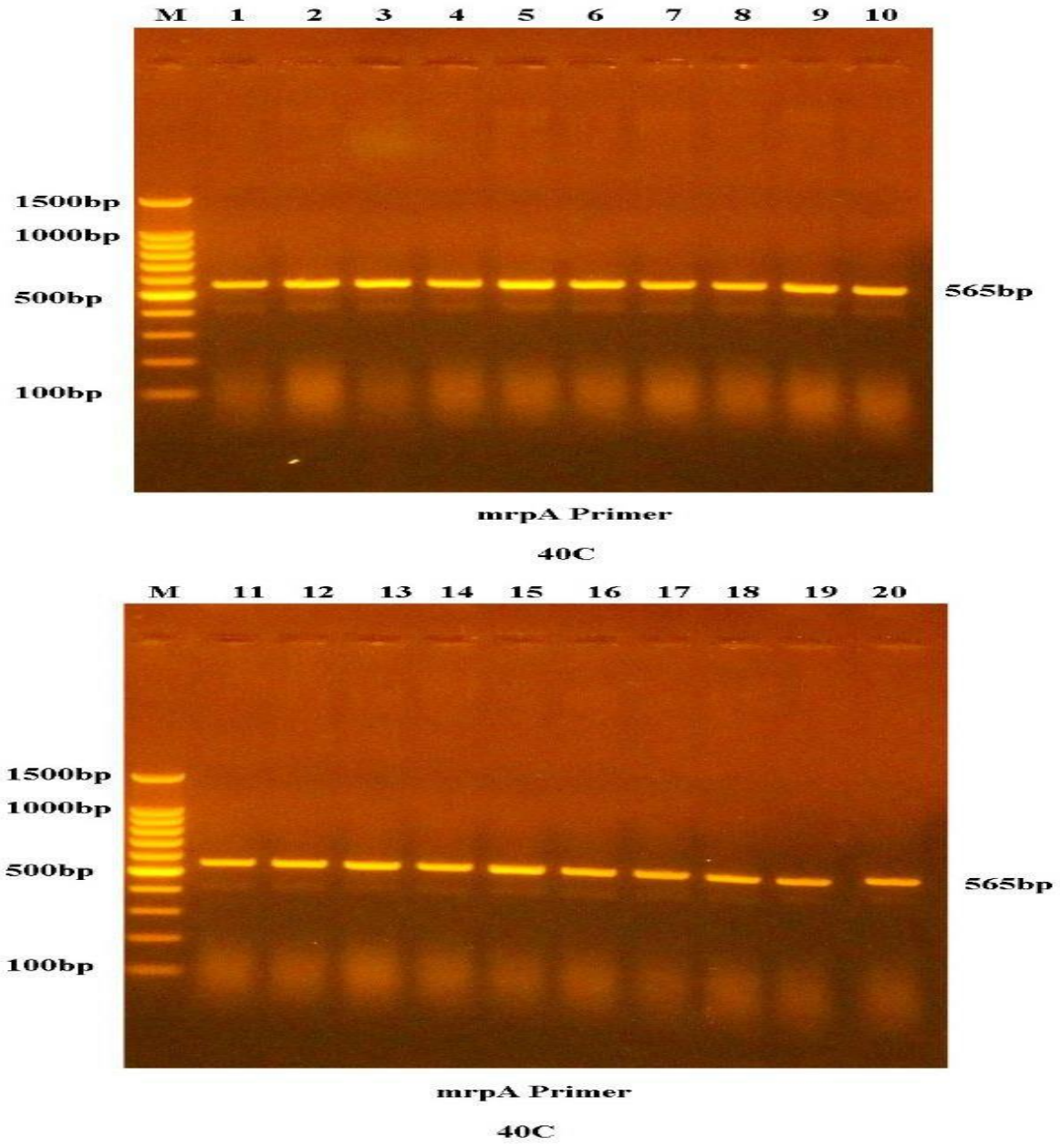

Figure3.3 . illustrations gel electrophoresis of *mrp*A gene that the positive result presents in 20 isolates . M : Ladder with (1500bp) and agarose 2% at a voltage difference of 100 volts for 60 minutes.

The result of the study showed that *fli*C gene appeared in 20(100%) . The result of electrophoresis showed of the present of a segment of length 1095bp belonging to the fliC gegn . This result was shown in figure (4).

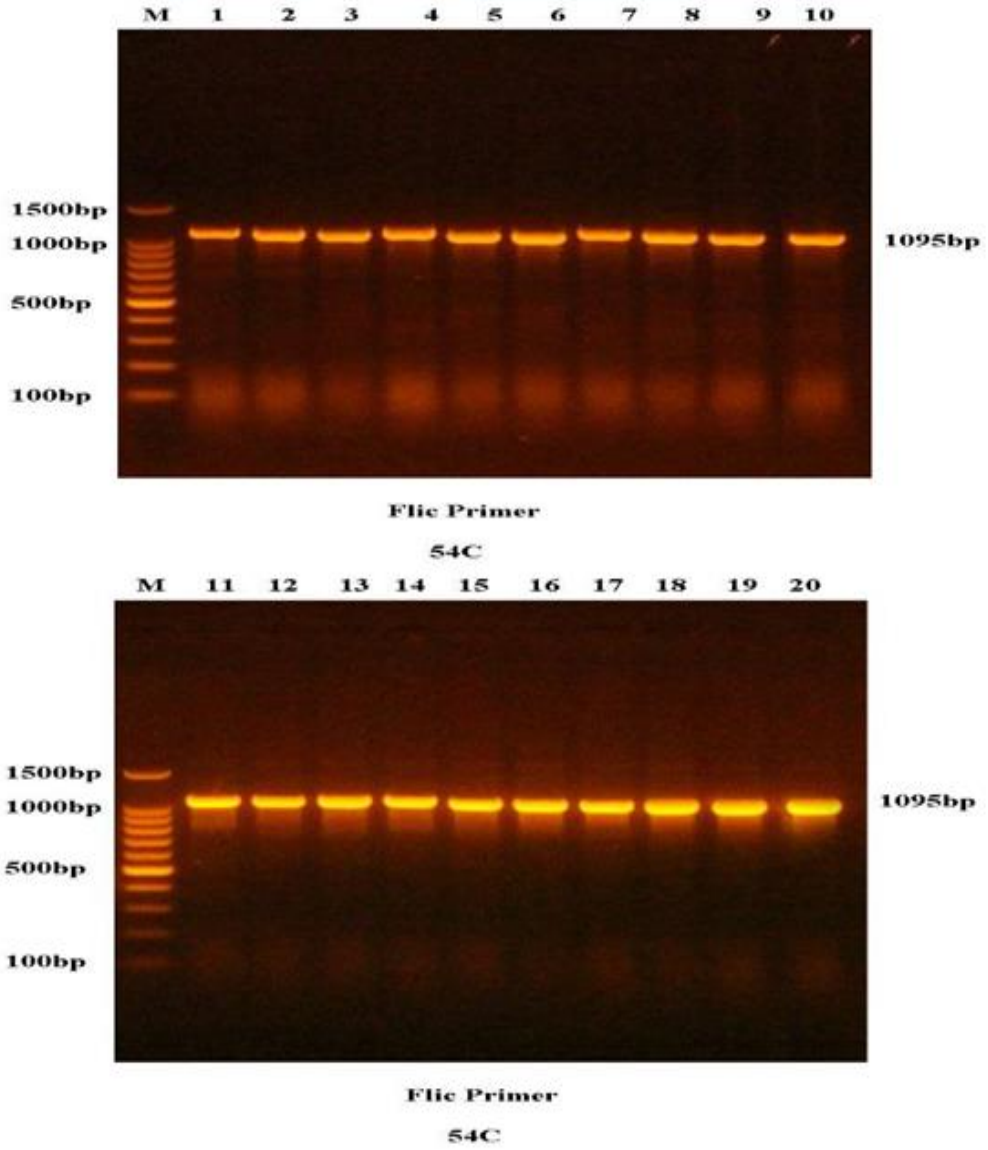

Figure 3. 4 . . Showed the electrophoresis of the *fliC* gene and the appearance of the gene in all isolates. M: Ladder with (1500bp) and agarose 2% at a voltage difference of 100 volts for 60 minutes.

DISCUSSION

The study showed that proteus bacteria have varying resistance to antibiotic , as the percentage of resistance to Amoxicillin-Clavulanic acid 85% the results arranged with [20] *P. mirabilis* resistance of Amoxicillin-Clavulanic acid was 94.3% . The isolated of *P. mirabilis* resistance of cefotaxime was 80% the result arranged with [21,22] *P. mirabilis* resistance of cefotaxime was 90% and 87% . The resistance of *P. mirabilis* isolates to Trimethoprim was 80% the results arranged with [23,24] *P. mirabilis* resistance was 72% and 79.16% . *P. mirabilis* resistance to Piperacillin 30% the result agreed with [25,26] the isolate resistance was 20% and 40% ,While the isolates not agree with [27,28] isolate resistance was 78.4% and 75% . Isolates resistance to Ciprofloxacin was 20% the result agree with [28, 29] . *P. mirabilis* isolate resistance to Imipenem was 25% The result arranged with[28,29] resistance was 16.2% , 25%. The resistance of isolates to Amikacin was 25% the result similar with [28,30] the resistance was 16.6% . It was found that isolates of *P. mirabilis* resistance to most antibiotics that are widely used, and the reason for this may be attributed to the bacteria's adaptation to the hospital environment, and the regular use of antibiotics works to kill the bacteria present in the environment while the bacteria remain inside . Antibiotics continuously, which allows the occurrence of mutations in the genetic material, resistance is also attributed to the possession of these bacteria for plasmids or Resistance factors (RF), which can be transferred from resistant to sensitive isolates to become resistant to one or more antibiotics. In the present study, 100% of strains had the rsbA gene, The results which agreed with [20,21] who found that 100% , 94.3% respectively . The phosphotransfer intermediate rsbA is encoded by the rsbA gene, which is part of the phosphorelay system. The rsbA gene, which regulates swarming behavior, has been identified as a swarming

repressor. The *fla*A and *fli*C found in isolates 100% The present study was agreed with the result of [30, 31, 32, 33,34] that displayed all isolate of *P. mirabilis* have gene *fli*C and *fla*A in the rate of (100%) . The flaA and flaB gene have an 80% homologous DNA sequence, so recombination can occur between them and then produce occasional hybrids that enable them to evade the host's immune system during the infection process . The process of regulating the production of the Flagelin protein is carried out by a gene symbolized by flhDC, which is a Class1 gene that codes for a heterothetrameric quaternary protein complex called FlhD2C2 that regulates the expression of the additional genes required for the differentiation of swimming cells by activating the Class2 genes that code for the basel body and flagellum proteins, the 28ð factor activates the Class3 genes necessary for the synthesis and synthesis of the Flagellin protein[35] .

The mrpA gene found in 100% of isolates the result was agree with of [33,32] that *mrp*A genes found in (100%) isolate of *P. mirabilis*. The MR/P type is one of the most important types of fimbria and encodes a number of chromosomal genes carried in two copies, the first version in the form of an operon and includes nine mrpABCDEFGHJ (mrp operon) genes required to synthesize and assemble the fimbra, while the second version is in the form of a single gene, mrpI, which regulates the reproduction of operon. The first step of urolithiasis is the accumulation of *P.mirabilis* bacteria in the lumen of the bladder, which increases the deposition of calcium ions, and this requires the presence of the enzyme urease and fimbria MRP [30,34,31] .

CONCLUSIONS

1- Possession of *P. mirabilis* isolates Many virulence factors that increase its pathogenicity.
2- The resistance of the isolates to one antibiotic from three different groups gives it the characteristic of multiple resistance, wherever it was all MDR isolates possess multiple resistance, and no isolate showed resistance to all antigens .The highest percentage of resistance towards Doxycycline antagonists, while Amikacin and Impenem antagonists were shown One of the most effective antibiotics against the tested isolate .
3- Twenty isolates possessed genes encoding flagella *fla*A, *fli*C, villi *mrp*A, swarmming genes *rsb*A, *wos*A.

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REFERENCES

1. **Hasan T, Alasedi K, Jaloob A (2021): Proteus Mirabilis Virulence Factors.** https://www.bing.com/search?q=Proteus+Mirabilis+Virulence+Factors&qs=n&form=QBRE&sp=-1&ghc=1&pq=proteus+mirabilis+virulence+factors&sc=10-35&sk=&cvid=5BCC3D7D49BB42FCAF579EF6E03064FB&ghsh=0&ghacc=0&ghpl=
2. **Hasan T, Al-Harmoosh R(2020):** Mechanisms of antibiotics resistance in bacteria. Sys Rev Pharm., 11(6): 817-823. **https://www.bing.com/search?q=Mechanisms+of+antibiotics+resistance+in+bacteria&qs=n&form=QBRE&sp=-1&pq=mechanisms+of+antibiotics+resistance+in+bacteria&sc=0-48&sk=&cvid=6BC9947E4ABC4A3181E99CF7EA6B41DB&ghsh=0&ghacc=0&ghpl=**
3. **Dougnon, V, Assogba P, Anago E (2020):** Enterobacteria responsible for urinary infections: a review about pathogenicity, virulence factors and epidemiology. Journal of Applied Biology and Biotechnology , 8(01):117-124.
4. **Abd Sharad A, Almuharib O, Hussein N (2020):** Effect of Xanthium Strumarium Extract on Some Virulence Factor of Proteus Mirabilis Isolated from Patients in Ramadi Hospital. Systematic Reviews in Pharmacy, 11(9): 1122-1124.
5. **Hasan J (2021):** Assessment of Probiotic Activity on Adhesin genes Expression of Aquatic and Clinical Klebsiella pneumoniae Isolates, PhD thesis. college of science. Baghdad University. https://www.bing.com/search?q=Assessment+of+Probiotic+Activity+on+Adhesin+genes+Expression+of+Aquatic+and+Clinical+Klebsiella+pneumoniae+Isolates&qs=n&form=QBRE&sp=-1&pq=assessment+of+probiotic+activity+on+adhesin+genes+expression+of+aquatic+and+clinical+klebsiella+pneumoniae+isolates&sc=8-115&sk=&cvid=60260E53010A49DB9F97CA998AB4A686&ghsh=0&ghacc=0&ghpl=
6. **Bunyan I, Albakery S (2020):** Molecular Detection of Some Virulence Factors of Uropathogenic Proteus mirabilis. https://www.researchgate.net/profile/Ilham-Bunyan/publication/351816163_Bunyan_Albakery_2021_Molecular_detection_of_virulence_factors_in_P_mirabilis_infection_Molecular_Detection_of_Some_Virulence_Factors_of_Uropathogenic_Proteus_mirabilis/links/60aba920299bf1031fc4a334/Bunyan-Albakery-2021-Molecular-detection-of-virulence-factors-in-P-mirabilis-infection-Molecular-Detection-of-Some-Virulence-Factors-of-Uropathogenic-Proteus-mirabilis.pdf
7. **Sedláková M, Urbánek K, Vojtová V *et al.* (2014):** Antibiotic consumption and its influence on the resistance in Enterobacteriaceae. BMC research notes, 7(1): 454.
8. **Anbuvannan M, Ramesh M, Viruthagiri G *et al.* (2015):** Synthesis, characterization and photocatalytic activity of ZnO nanoparticles prepared by biological method. Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy,143: 304–308.
9. **Jayaseelan S, Ramaswamy D, Dharmaraj S (2014):** Pyocyanin: Production, applications, challenges and new insights. World Journal of Microbiology and Biotechnology, 30(4): 1159–1168.
10. **Kaur M, Sharma S, Bedi P (2015):** Silica supported Brönsted acids as catalyst in organic transformations: A comprehensive review. Chinese Journal of Catalysis, 36(4): 520-549.
11. **Jones N, Ray B, Ranjit, K *et al.* (2008):** Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms. FEMS Microbiology Letters, 279(1): 71–76.

12. **Lee N, Lim C, Kim T *et al.* (2017):** Which hazard category should specific nanomaterials or groups of nanomaterials be assigned to and how. WHO-FWC-IHE-17.4-eng.pdf.
13. **Holt J, Kreig N, Sheath P *et al.* (1994):** Bergey's Manual of Determinative Bacteriology. https://agris.fao.org/agris-search/search.do?recordID=XF2015028636
14. **Procop G, Church D, Hall G *et al.* (2016):** Koneman's color atlas and textbook of diagnostic microbiology. https://books.google.com/books?hl=ar&lr=&id=HF3sDwAAQBAJ&oi=fnd&pg=PP1&dq=14-+Procop++G,+Church+D,+Hall+G++et+al.++(2016):+Koneman%27s+color+atlas+and+textbook+of+diagnostic+microbiology.+7thed.+Lippincott+Williams+and+Willkins.+Philadelphia.+Baltimore.+New+York.+London.&ots=rE9soZxDw9&sig=xZP681Op8JWib5K8FO5yO6PoR20
15. **Bauer A, Kirby W, Sherris J *et al.* (1996):** Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45(4): 493-496.
16. **Sosa V, Schlapp G, Zunino P (2006)**. Proteus mirabilis isolates of different origins do not show correlation with virulence attributes and can colonize the urinary tract of mice. Microbiology, 152(7): 2149-2157.
17. **Ali H, Yousif M (2015):** Detection of some virulence factors genes of Proteus mirablis that isolated from urinary tract infection. International Journal of Advanced Research , 3: 156-163.
18. **Badi S, Nowroozi J, Sepahi A (2014):** Detection luxS, qseC and rsbA genes' bands in Proteus mirabilis and Escherichia coli isolated from urinary tract infections. Pajoohandeh Journal, 19(3): 142- 147.
19. **Mirzaei, A, Habibi M, Bouzari S *et al.* (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. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6938180/
20. **Gupta A, Mumtaz S, Li C *et al.* (2019):** Combatting antibiotic-resistant bacteria using nanomaterials. Chemical Society Reviews, 48(2): 415-427.
21. **Al-Hamdani H, Al-Hashimy A (2020):** Molecular Detection Of urec, hpma, rsba and mrpa genes of Proteus mirabilis Urinary Tract Infection In Patient With Rheumatoid Arthritis.The Iraqi Journal of Agricultural Science, 51: 245-251.
22. **Al–Atbi A (2020):** Molecular study for one gene responsible for biofilm formation and the effect of some biofilm inhibitors in proteus mirabilis isolated from Burns and Wounds . MS thesis. college of science. Baghdad University.https://www.bing.com/search?q=Molecular+study+for+one+gene+responsible+for+biofilm+formation+and+the+effect+of+some+biofilm+inhibitors+in+proteus+mirabilis+isolated+from+Burns+and+Wounds+healing&qs=n&form=QBRE&sp=-1&ghc=2&pq=molecular+study+for+one+gene+responsible+for+biofilm+formation+and+the+effect+of+some+biofilm+inhibitors+in+proteus+mirabilis+isolated+from+burns+and+wounds+&sc=9-157&sk=&cvid=619AD9112082402898431732010BEAFD&ghsh=0&ghacc=0&ghpl
23. **Singla P, Sangwan J, Garg S *et al.* (2015):** Prevalence and Antibiogram of Multidrug resistant Uropathogenic Isolates of *Proteus mirabilis* in a Teaching Tertiary Care Hospital. International Journal of Current Microbiology Applied Sciences, 4(12): 675-682.
24. **Al-Bassam W, Al-Kazaz A (2013):** The isolation and characterization of Proteus mirabilisfrom different clinical samples. Journal of Biotechnology Research Center, 7(2):24-30
25. **Latif B, Zayn S, Ahmed I (2017):** A Comparative Bacteriological and Molecular Study on Some Virulence Factor of *Proteus* spp Isolated from Clinical and Environment Specimen. International Journal of Current Research and Academic Review, 5(5): 26-33.
26. **Wasfi R, Hamed S, Abd Allah N *et al.* (2020):** Proteus mirabilis biofilm: Development and therapeutic strategies. Frontiers in cellular and infection microbiology, 10: 414.
27. **Al-Jumaily E, Zgaer S (2016):** Multidrug resistant Proteus mirabilis isolated from urinary tract infection from different hospitals in Baghdad City. Int. J. Curr. Microbiol. App. Sci., 5(9): 390-399.
28. **Wang J, Chen P, Chang S *et al.* (2014):** Antimicrobial susceptibilities of Proteus mirabilis: a longitudinal nationwide study from the Taiwan surveillance of antimicrobial resistance (TSAR) program. BMC infectious diseases, 14(1): 486.
29. **Kadhim A, AL-Mathkury H, Obaid H (2014):** Role of Proteus mirabilis DNA in Comparison to Candida albicans DNA in Rats' Joints Infection. Iraqi Journal of Science, 55(3B): 1170-1182.
30. **AL- Dulaimi M(2019):** Detection of Some Virulence Genes of *Proteus mirabilis* Isolated From Different Infectious in Baquba City .MS thesis college of science. Diyala University . https://www.bing.com/search?q=Detection+of+Some+Virulence+Genes+of+Proteus+mirabilis+Isolated+From+Different+Infectious+in+Baquba+City+&qs=n&form=QBRE&sp=-1&ghc=1&pq=detection+of+some+virulence+genes+of+proteus+mirabilis+isolated+from+different+infectious+in+baquba+city+&sc=10-105&sk=&cvid=4E68926AC8E342868728F3EBE742A7A2&ghsh=0&ghacc=0&ghpl=
31. **AL-Oqaili N, AL-Shebli M, Almousawi A (2017**): Antimicrobial susceptibility and molecular characterization for some virulence factors of Proteus Mirabilis isolated from patients in AlQadisiyah Province/Iraq. Al-Qadisiyah Journal of Veterinary Medicine Sciences, 16(2): 1-9
32. **Al-Mayahi F (2017):** Phenotypic and Molecular detection of Virulence factors in Proteus mirabilis isolated from different clinical sources. Bas J Vet Res., 16(1): 369-88**.**
33. **Sosa V, Schlapp G, Zunino P (2006)**: Proteus mirabilis isolates of different origins do not show correlation with virulence attributes and can colonize the urinary tract of mice. Microbiology, 152(7): 2149-2157.
34. **Mirzaei A, Habibi M, Bouzari S *et al.* (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: 3967.
35. **Umpierreze A. Scavone P. Romanin D. Alejandre J. Chabalgoity J. Rumbo M. and Zunino P. (2013):**Innate immune responses to *Proteus mirabilis* flagellin in the urinary tract. https://www.bing.com/search?q=Innate+immune+responses+to+Proteus+mirabilis+flagellin+in+the+urinary+tract&qs=n&form=QBRE&sp=-1&pq=innate+immune+responses+to+proteus+mirabilis+flagellin+in+the+urinary+tract&sc=5-75&sk=&cvid=C025CA0A69F847298324BCFB5133674E&ghsh=0&ghacc=0&ghpl=