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ORIGINAL ARTICLE

Molecular Investigation of bla*TEM*, *sul1* and *papG* genes and Molecular Phylogenetic of Beta- lactamase *TEM* Gene in Multidrug Resistance *Proteus mirabilis* in Baquba city/ Iraq

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Key words:
P. mirabilis, blaTEM, sul1, papG, phylogenetic tree

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ABSTRACT

Background: *Antimicrobial resistance in Proteus mirabilis is increasing worldwide, such as the resistance to sulfonamides and beta lactams due to the production of extended-spectrum β-lactamases (ESBL) in hospitalized patients.* ***Objective:*** *This study aimed to detect some antibiotic resistance genes of P. mirabilis bacteria causing various infections using PCR.* ***Methodology:*** *One hundred eighty specimens were collected from different clinical samples in Diyala. Antibiotics resistance and some virulence factors were detected. PCR was done for blaTEM, sul1 and papG genes and screened through uniplex PCR then sequencing and phylogenetic tree of blaTEM gene was established.* ***Results:*** *Twenty (18.18%) isolates of P. mirabilis were obtained. All P. mirabilis isolates were phenotypically positive (100%) for extracellular urease, swarming and biofilm formation. Antibiogram showed resistance towards Trimethoprim 95% followed by Ampicillin and Amoxicillin-Clavulanic acid 85% and 80% respectively. Ciprofloxacin and Gentamicin showed 35% resistance, while Levofloxacin and Amikacin showed 5% resistance. Ceftriaxone, Azithromycin and Cefixime showed 70%, 65% and 60% respectively. All isolates were sensitive to Meropenem. Isolates showed that 100% of isolates had sul1 gene, while papG gene was positive in 95% of isolates and finally blaTEM gene was found in 80% of isolates. blaTEM sequencing showed a similarity of 99% with the reference strains of P. mirabilis bacteria.* ***Conclusion:*** *P. mirabilis bacteria were resistant to several antibiotics, especially trimethoprim and ampicillin. Meropenem, followed by amikacin, was the most effective antibiotic against all isolates. The sul1 gene was the most common resistance gene, and the prevalence of blaTEM and papG genes was low.*

INTRODUCTION

Proteus mirabilis is an opportunistic pathogen that can cause serious invasive diseases in chronically ill, elderly, or pregnant individuals. It is a major cause of hospital-acquired infections[1]. *P. mirabilis* can cause urinary tract infections, respiratory tract infections, wounds, burns, and gastrointestinal infections[2]. This widespread infection caused by *P. mirabilis* is due to the fact that it contains several virulence factors, including adhesion, toxins, flagella, production of enzymes such as urease, biofilms, and a highly resistant phenotype to antibiotics[3]. Biofilm production is an important resistance mechanism because it promotes resistance gene transfer, renders bacterial colonies resistant to antibiotics, increases antibiotic metabolism, and enhances the expression of an efflux pump[4]. During phagocytosis, it protects bacteria from antibody opsonization[5].

There are several virulence genes that help *P. mirabilis* survive in the urinary tract such as urease, hemolysin, villi, and flagella. However, *P. mirabilis* strains differ in the range and levels of expression of virulence genes that can affect bacterial growth and persistence in urinary tract[6]. *P. mirabilis* genome has many genes which encode proteins that is responsible for the resistant of antibiotic and the development of multidrug and widespread resistance strains[7]. Exceeding 130 integron gene cassette arrays of diverse antibiotic resistance genes have been found; these genes that has resistance are commonly prevalent on plasmids and integrons, resulting in fast transmission and treatment failure[8].

blaTEM genes significantly correlated with the resistance to piperacillin/ tazobactam obtained by the disk diffusion method these enzymes consider primary causal agents that lead to increased resistance against the β-lactam group, especially cephalosporins antibiotics that include (cefotaxime, ceftazidime), and aztreonam[9]. The study aimed to investigated beta-lactamase production and prevalence of *blaTEM*, *sul1* and *papG* genes in *P. mirabilis* isolated from different sources in Diyala, Iraq

METHODOLOGY

Isolation and identification of *P. mirabilis*

The collection of 180 specimens from various clinical sources (urine, stool, otitis media, wounds, and vaginitis), was supervised by a specialist physician from males and females in different ages at Baquba Teaching Hospital and Al-Batoul Maternity and Children's Hospital in Diyala Governorate/Iraq, during September 2024 to December 2024. For isolation and preliminary diagnosis, collected samples were cultured on McConkey, blood, and nutrient agar media after making sure that patient had not taken antibiotics for at least three days. *P. mirabilis* isolates were identified using biochemical tests (Indole, Oxidase, Catalase, Urease, Methyl Red, Citrate), microscopic analysis (Gram's stain), and morphological characteristics on culture media. VITEK2 System was used to confirm the diagnosis.

Antibiotic susceptibility test (AST)

The method of Bauer et al.[10] was used to test the sensitivity of *P. mirabilis* isolates using eleven antibiotics discs.

Biofilm formation

The biofilm formation test was carried out according to Ghellai's et al.[11] process as following:

Twenty-five µl of overnight *P. mirabilis* were incubated in flat-bottom- microtiter plate with 180µl of Brain Heart infusion (BHI) broth and sucrose (2%). The plate was covered with a lid and incubated aerobically (24–30 hrs. at 35–37°C). The contents of the wells were decanted, and washed three times with regular saline pH=7.2 to extract unattached bacterial cells. The plates were then drained inverted and fixed with 150 ml methanol then dried for 15 min. at room temperature. About 200µl of crystal violet solution (0.1%) were added for 15 min, then washed and dried at room temperature. Extracted with 200µl of 95 % ethanol, for dye resolubilizing. ELISA reader was used, the absorbance was calculated at 630 nm. All of the test OD values were subtracted from the control well's OD value, and the results were as follows:

OD ≤ ODc = no biofilm producer;
ODc < OD ≤ 2 ODc= weak biofilm producer;
2 ODc < OD≤ 4 ODc = moderate biofilm producer; 4 ODc < OD= strong biofilm producer

Swarming motility

This test was conducted to investigate whether the isolates under study have the phenomenon of swarming. Blood agar medium was inoculated at its center with 10µl of bacterial suspension, then incubated for 18-24 hrs. at 37°C. The result is considered positive when a wave movement appears on the surface of the plates[12]

Hemolysin production

The bacteria were inoculated onto blood agar with 5% human blood and incubated at 37°C for 24 hrs. After incubation, total hemolysis formed around the cultured colonies indicating a positive test[13].

Urease production

The color change of urea agar medium after inoculated with *P. mirabilis* colonies and incubated at 37°C for 24 h from yellow to purple is evidence of positive results[13].

DNA extraction and screening for targeted genes

Twenty isolates under study were amplified for detection of some virulence genes (*blaTEM*, *sul1* and *papG* genes). For extracting the genomic DNA, extraction kit (Promega/USA) was used according to the manufacture instruction. List of the used primers are summarized in Table 1. PCR reaction was carried out in 20µl of Premix (Promega, USA). The mixture consists of 12.5µL master mix, 1µL of each primer, 5.5µL nuclease free water, 5µL template DNA. The thermal profile included initial denaturation at 95°C for 3 min, followed by 35 cycles of 95°C (60 s) of denaturation, 62°C (90s) of annealing and 72°C (60s) and final extension of 72°C (7 min). PCR products were electrophoresed in 1.5% gel agarose in 1X TBE buffer with staining of 1µL of each loading buffer and gel red and then observed under UV emission. Finally, the sizes of the PCR products were determined by comparing them with the migration of the DNA ladder[14, 15, 16].

Table 1: Target genes and sequences of primers

Genes	Nucleotide sequences (5′–3′)	Product size bp
blaTEM	F: CGC CGC ATA CAC TAT TCT CAG AAT GA R: ACG CTC ACC GGC TCC AGA TTT AT	445
Sul1	F-TGGTGACGGTGTTCGGCATTC R-GCGAGGGTTTCCGAGAAGGTG	798
PapG	F: GGGATGAGCGGGCCTTTGAT R: CGGGCCCCCAAGTAACTCG	190

Table 2: PCR program

Amplified gene	Primary denaturation	cycles	Denaturation	Annealing	Elongation	Final elongation
Sul1	**95°C/ 3min**	**35**	**95°C/ 60 sec**	**55°C/60 sec**	**72°C/60 sec**	**72°C/2min**
TEM	**95°C/ 3min**	**35**	**95°C/ 60 sec**	**62°C/90 sec**	**72°C/60 sec**	**72°C/2min**
PapG	**95°C/ 3min**	**35**	**95°C/ 60 sec**	**63°C/30 sec**	**72°C/60 sec**	**72°C/3min**

Sequence Analysis

Sequencing of the *blaTEM* gene was performed for 5 isolates under study and PCR products were sent by automated DNA sequencer from Macrogen Corporation, Korea. The results were received by e-mail and analyzed using Geneus software.

Phylogenetic tree construction

A specific phylogenetic tree was established in this study based to on neighbour-joining protocol. The differences were compared with their neighbour homologous reference sequences using the NCBI-BLASTn server[17].

RESULTS

Isolation and identification of *p. Mirabilis*

Twenty isolates of *P. mirabilis* were obtained with an infection rate of (18.18%). While other growing bacterial species constituted 90 (81.82%), as shown in figure 1. The isolates were positive for catalase, methyl red and urease tests, while they were negative for indole, and oxidase, and differed in their ability to consume citrate and motility.

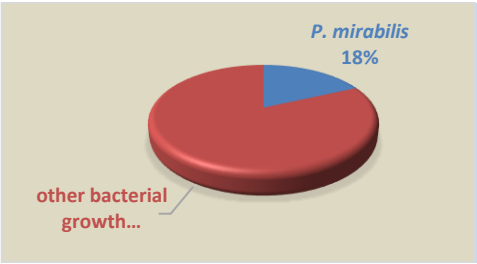

Figure 1: Isolation rate of bacterial growth

Antibiogram

Twenty of *P. mirabilis* isolates were tested against 11 antibiotics, as shown in Figure 2. *P. mirabilis* isolates showed varying degrees of resistance to different antibiotics. A high resistance to Trimethoprim 95% followed by Ampicillin and Amoxicillin-Clavulanic acid 85% and 80% respectively. Ciprofloxacin and Gentamicin showed 35% resistance, while Levofloxacin and Amikacin showed 5% resistance. Ceftriaxone, Azithromycin and Cefixime showed 70%, 65% and 60% respectively. All isolates were sensitive toward Meropenem.

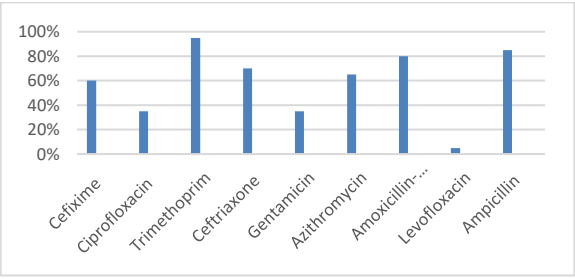

Fig. 2: Antibiogram of *P. mirabilis* isolates

The majority of the isolates were found to be multidrug resistant (MDR) as they were resistant to three or more antibiotics (Figure 3). In this study, the prevalence of multidrug resistant *P. mirabilis* was high. It was found that 15 (75%) of all isolates were multidrug resistant (MDR), while only five isolates tended to be XDR (25%) while no PDR was detected.

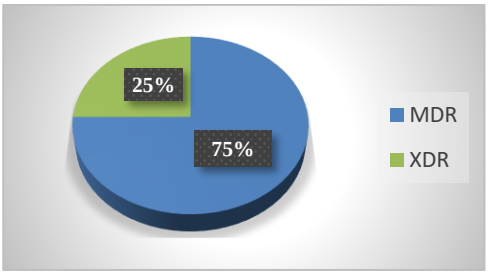

Fig. 3: Multiple resistance of the isolates under study

Virulence factors of *P. mirabilis*

The results of the current study showed that all isolates were 100% motile with swarming and non-hemolysin producer on blood agar, they were observed in the form of concentric rings covering surface of the medium and moving towards the edge of the dish. The present results showed that all *P. mirabilis* isolates were phenotypically positive (100%) for extracellular urease; and all isolates (100%) had the ability to form biofilms at medium degree.

DNA extraction and purification

Twenty *P. mirabilis* isolates were subjected to amplification of virulence genes (*blaTEM*, *sul1* and *papG* genes) based on their possession of the most potent virulence factors. Accordingly, genomic DNA was extracted using an extraction kit (Alpha DNA, USA). The concentrations and purity of DNA were measured by Nano Spectrometry. All samples had DNA concentrations ranging from (62.1-12.5) ng/µl, while the purity ranged from (1.8-1.98). DNA from the six isolates was subjected to 1.5% agarose gel electrophoresis to confirm the integrity of the DNA. The results showed unique discrete bands indicating successful isolation of pure DNA.

***papG* gene detection**

The results showed that 19 (95%) of the isolates contained the *papG* gene (Figure 4).

Detection of *sul1* gene

The results of the current study showed that all 20 (100%) isolates were carriers of the *sul1* gene. The results of electrophoresis showed the presence of a band of 798 base pairs long belonging to *sul1* gene when compared with the standard size guide DNA ladder, Figure (5).

Detection of *blaTEM* gene

blaTEM gene was amplified and the presence of *blaTEM* gene was screened in (20) isolates and confirmed using single-stranded polymerase chain

reaction as a gold standard method. A single band was observed at a specific molecular weight (445 base pairs). The results of the current study illustrated in figure (6) showed that 16 (80%) of the studied isolates contained the *blaTEM* gene.

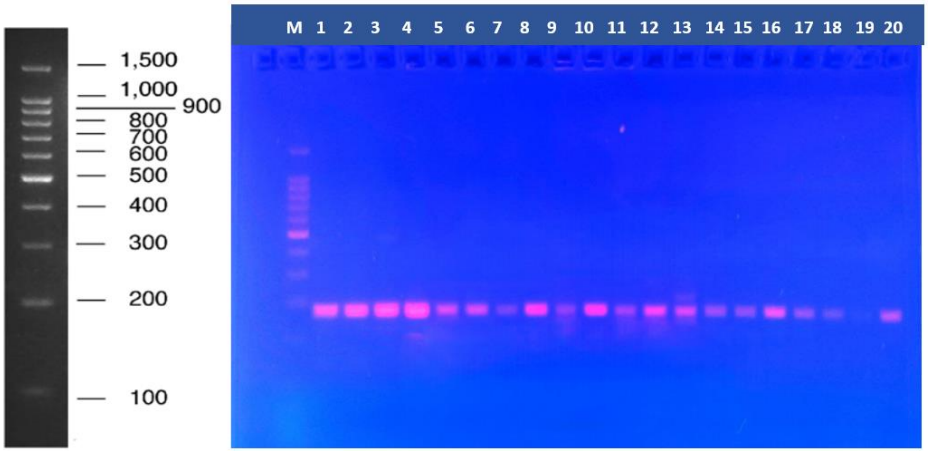

Fig. 4: Agarose gel electrophoresis (1.5% agarose, 7 V/cm2 for 60 min) for papG gene (amplicon 190 bp); size ladder 100 bp.

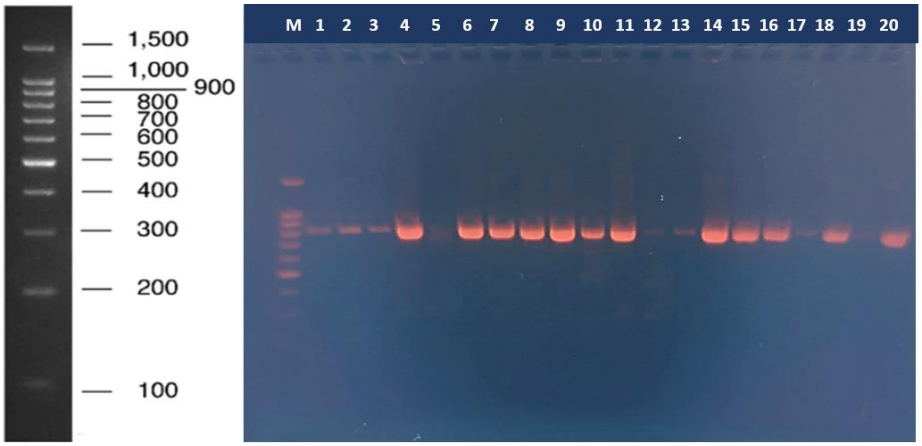

Fig. 5: Agarose gel electrophoresis (1.5% agarose, 7 V/cm2 for 60 min) of *Sul1* gene (amplicon 798 bp long) and 100 bp long volume ladder.

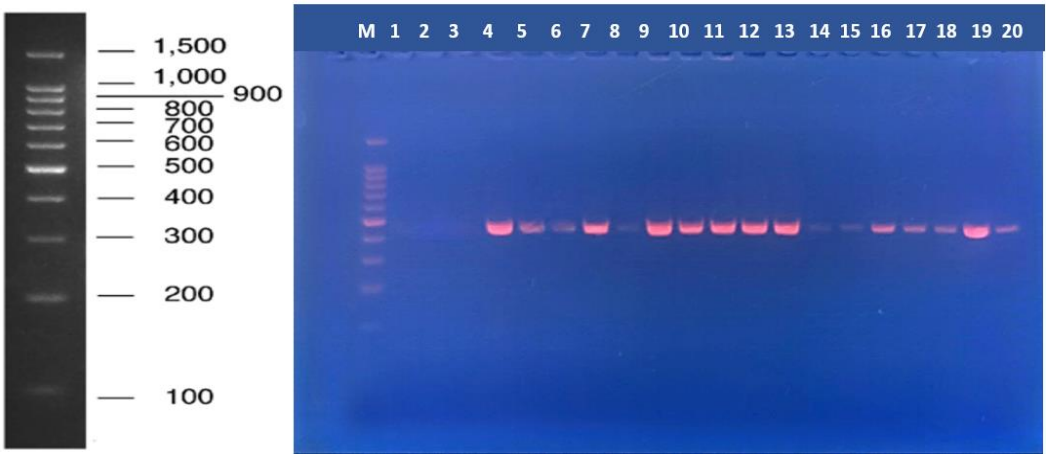

Fig. 6: Agarose gel electrophoresis (1.5% agarose, 7 V/cm2 for 60 min) for *TEM* gene (amplicon 445 bp) size ladder 100 bp.

DNA sequence

Five isolates of *Proteus mirabilis* were selected for DNA sequencing analysis, the isolates that were selected were isolates No. 4, 9, 10, 18 and 20, as these isolates have high resistance to most of the antibiotics under study and were classified as MDR as they resisted 7 antibiotics more than 3 different families, sequencing experiments for a specific length fragment using the NCBI blastn engine indicated a similarity of 99% with the reference strains of *P. mirabilis* bacteria. By comparing DNA sequences of these local samples with the DNA sequences extracted from the NCBI website (GenBank acc. CP046048.1), the exact locations and other details of these PCR fragments were determined, as well as the locations of the forward and reverse primers, respectively, in both targeted fragments, Figure (7).

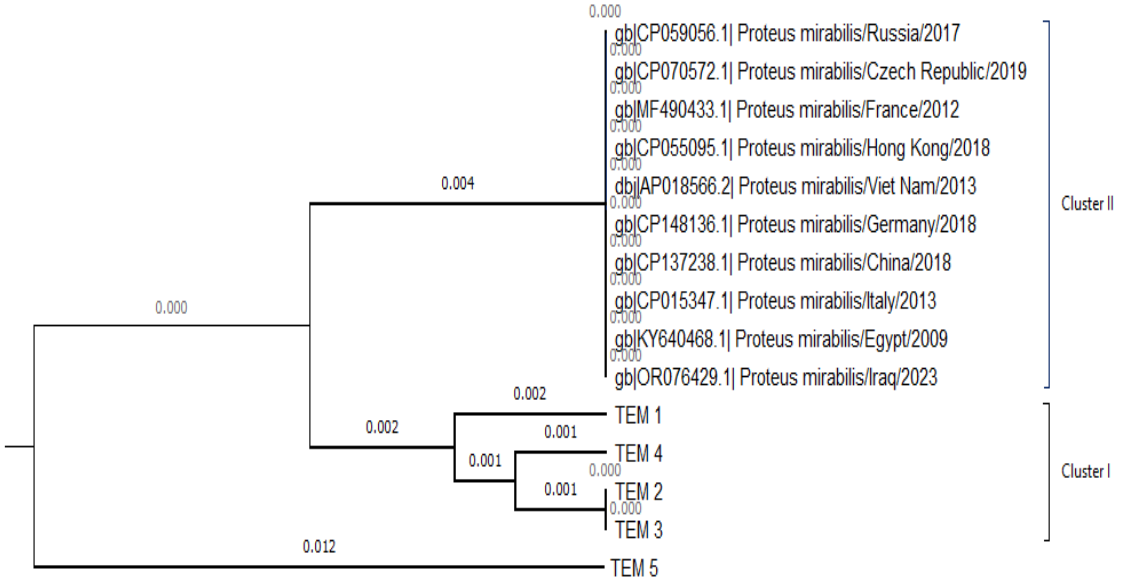

Fig. 7: Phylogenetic tree constructed based on the 445 bp amplified fragment used to amplify a portion of the *blaTEM* gene in Proteus mirabilis.

1. Nodes and Labels:

The phylogenetic tree consists of nodes representing sequences labeled as:

TEM sequences: #TEM_1 to #TEM_5 may indicate distinct β-lactamase variations. Isolates from various countries: These sequences come from France, Russia, the Czech Republic, China, Germany, Vietnam, Hong Kong, Egypt, Italy, and Iraq. The Iraqi isolate gb|OR076429.1| is identified as the outgroup.

2. Distance Matrix:

The evolutionary distances between every pair of sequences are displayed in the matrix. The distances show evolutionary divergence and range from 0.0000 (identical sequences) to values like 0.0181.

Tree Interpretation

1. Clustering of TEM Sequences:

The TEM sequences are relatively similar to one another:

TEM_1, TEM_2, and TEM_3 have a distance of 0.0051, suggesting close evolutionary relationships. TEM_4 is slightly diverged with a distance of 0.0025 from TEM_1, TEM_2, and TEM_3. TEM_5 shows the most divergence within the TEM group with distances ranging from 0.0155 to 0.0181.

2. Outgroup Placement:

The Iraqi isolate (gb|OR076429.1|) serves as the outgroup. It shows a moderate divergence (~0.0051–0.0103) from the TEM sequences, indicating that it is distantly related but still comparable.

3. Global Isolates:

Isolates from Egypt, Italy, China, Germany, Vietnam, Hong Kong, France, Russia, and the Czech Republic show zero divergence (distance = 0.0000) among themselves, forming a monophyletic clade. This suggests they are highly similar or identical in terms of the sequences analyzed. This clade diverges from the TEM sequences by a moderate distance (~0.0051–0.0181).

4. Evolutionary Patterns:

The global isolates are a highly conserved group, which could suggest that a clonal lineage propagated over a variety of geographic locations between 2009 and 2019. Because TEM sequences are more varied, they might correspond to different evolutionary paths,

perhaps as a result of functional adaptations (such as resistance mechanisms). A closely linked clade of worldwide isolates that suggests recent evolutionary divergence is revealed by the phylogenetic tree. TEM_5 is the most distinctive of the more varied TEM sequences.

DISCUSSION

The isolation results were consistent with the results of many international studies, which confirmed that the presence of these bacteria falls within a percentage of (5-18%) among cases of positive bacterial growth in clinical samples[18].

The development of antibiotic resistance is often linked to the overuse or misuse of prescribed antibiotics. Resistance to superbugs remains an important clinical therapeutic problem, as can be found in the increasing multidrug resistance of these bacteria. The emergence of high-level antibiotic resistance poses a growing threat to global health[19].

Multidrug resistance (MDR) may be caused by resistance factors present on chromosomes or mutations in a resident gene. A different observation by Ramatla et al.[20] revealed that (30.7%) of the isolates were classified as multidrug resistant (MDR). However, it may also expand through the acquisition of resistance genes through horizontal transfer[21]. The results of virulence factors were in consistent with Al-Atabi[22] at Baghdad Hospital, where he showed that 100% have the ability to move and form swarming phenomenon. It enables bacteria to adapt under suitable and unsuitable environmental conditions, as it succeeds in competing with other bacterial species in liquid media and solid surfaces because it has an advanced movement system[23]. The isolates were unable to produce extracellular hemolysin. The function of hemolysin is to form pores in target host cells. It has been described that hemolytic activity helps *P. mirabilis* to spread to the kidney during infection[24].

The results of biofilm production were in consistent with Mirzaei et al.[26] that all isolates were biofilm producers. The ability of bacteria to form biofilms has been associated with increased antibiotic resistance and chronic recurrent infections[27], and provide the survival of bacteria in different environments, thus enabling better adaptation to external conditions and more efficient utilization of nutrients[28].

In the current study (95%) of the isolates contained *papG* gene. This revealed the importance of Flagella as a virulence factor for *P. mirabilis*, helping it to move, attach, and thus cause infection. The flagellum consists of a basic protein known as flagellin[29].

blaTEM genes were significantly associated with piperacillin/tazobactam resistance obtained by disk diffusion method, and these enzymes are considered to be key causal factors leading to increased resistance against the beta-lactam group, especially cephalosporin antibiotics including cefotaxime, ceftazidime, and aztreonam[9]. In this study (80%) of studied isolated contained the *blaTEM* gene. The results of the current study differed from what was found by AL-Fatlawi et al.[30] in Kufa, where it was found that only 40% of the isolates contained the *blaTEM* gene.

A comprehensive and specialized phylogenetic tree was constructed for *blaTEM* gene based on the sequences of the DNA tested for this gene in the two analyzed fragments. Along the folded DNA sequences, this tree contained the tested isolates aligned with their close sequences in a pattern known as neighbor-joining mode. The current phylogenetic tree showed the presence of one major type of organism, *P. mirabilis*. Based on the analysis of genome sequences of *P. mirabilis*, the studied sequences were distributed into several adjacent evolutionary branches, indicating the presence of a wide range of genetic diversity of this organism.

CONCLUSIONS

Numerous *P. mirabilis* isolates in this study were resistant to the majority of the antibiotics used. Bacteria can resist antibiotics in a number of ways, including by producing enzymes that hydrolyze the antibiotics, such as Metallo β-lactamase and Extended Spectrum β-lactamase, formation of biofilm or by having other mechanisms to resist antibiotics. Various beta-lactamase and flagellar genes were detected among the *P. mirabilis* of *blaTEM*, *sul1* and *papG*. The gene of *sul1* was the most common resistance gene and there was low prevalence of *blaTEM* and *papG* genes.

Ethical Approval

Biology Department Local Committee agreed to the experiments mentioned in this study and all volunteers give formal consent and details and advantages were given to the patients based on research. The study was undertaken by University of Diyala/College of Pure science under supervision of Science Unite in the College.

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Declarations:

Consent for publication: Not applicable

Availability of data and material: Data are available upon request.

Competing interests: The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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REFERENCES

1. Hamilton AL, Kamm MA, Ng SC, Morrison, M. Proteus spp. as putative gastrointestinal pathogens. Clinical microbiology reviews, 2018, 31(3), 10-1128.
2. Smelov V, Naber K, Johansen TEB. Improved classification of urinary tract infection: future considerations. European Urology Supplements, 2016, 15(4), 71-80.
3. Aghapour Z, Gholizadeh P, Ganbarov K, Bialvaei AZ, Mahmood SS, Tanomand A, Kafil HS. Molecular mechanisms related to colistin resistance in Enterobacteriaceae. Infection and drug resistance, 2019, 965-975.
4. Cadavid E, Robledo SM, Quiñones W, Echeverri F. Induction of biofilm formation in Klebsiella pneumoniae ATCC 13884 by several drugs: the possible role of quorum sensing modulation. Antibiotics, 2018, 7(4), 103.
5. Seifi K, Kazemian H, Heidari H, Rezagholizadeh F, Saee Y, Shirvani F, Houri H. Evaluation of biofilm formation among Klebsiella pneumoniae isolates and molecular characterization by ERIC-PCR. Jundishapur journal of microbiology, 2016, 9(1).
6. Abbas KF, Jawad K, Khafaji AL, Maysaa S, Shukri AL. Molecular detection of some virulence genes in Proteus mirabilis isolated from Hillaprovince.Int.J.Res.Stud.in Biosci.(IJRSB), 2015. 3(10), 85-89.
7. Obadire SO, Mitsan O. IP, U. Prevalence and Antibiotic Susceptibility Pattern of Proteus Species Isolated from Clinical Specimens from Selected Hospitals in Jigawa State, North-West Nigeria. Sokoto Journal of Medical Laboratory Science, 2022, 7(4).
8. Hu R, Wang X, Muhamamd I, Wang Y, Dong W, Zhang H, Ma H. Biological characteristics and genetic analysis of a highly pathogenic Proteus mirabilis strain isolated from dogs in China. Front. Vet. Sci, 2020, 7, 589.
9. Li Z, Peng C, Zhang G, Shen Y, Zhang Y, Liu C, Wang F. Prevalence and characteristics of multidrug-resistant Proteus mirabilis from broiler farms in Shandong Province, China. Poult.sci, 2022, 101(4), 101710.
10. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol, 1996, 45(4): 493-496.
11. Ghellai L, Hassaine H, Klouche N, Khadir A, Aissaoui N, Nas F, Zingg W. Detection of biofilm formation of a collection of fifty strains of Staphylococcus aureus isolated in Algeria at the University Hospital of Tlemcen. J. Bacteriol. Res, 2014; 6(1), 1-6.
12. Liaw S.-J, Lai H.-C, Ho S.-W, Luh K.-T, Wang W.-B. Inhibition of virulence factor expression and swarming differentiation in Proteus mirabilis by p-nitrophenylglycerol. J. Med. Microbiol, 2000, 49, 725–731.
13. Al-Mayahi FS. Phenotypic and Molecular detection of Virulence factors in Proteus mirabilis isolated from different clinical sources. Bas J Vet Res, 2017, 16(1), 369-88.
14. Hafza N, Challita C, Dandachi I et al. Competition assays between ESBL-producing E. coli and K. pneumoniae isolates collected from Lebanese elderly: an additional cost on fitness. J Infect Public Health, 2018; 11: 393–7.
15. Saenz Y, Brinas L, Dominguez E, Ruiz J, Zarazaga M, Vila J, Torres C. Mechanisms of resistance in multiple-antibiotic-resistant Escherichia coli strains of human, animal, and food origins. Antimicrob Agents Chemother. 2004; 48(10):3996–4001.
16. Johnson JR, Russo TA, Brown JJ, Stapleton A. papG Alleles of Escherichia coli Strains Causing First-Episode or Recurrent Acute Cystitis in Adult Women. The Journal of Infectious Diseases, 1998, 177(1), 97–101.
17. Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S, Madden T. Primer-BLAST: A tool to design target-specific primers for polymerase chain reaction. BMC Bioinfo, 2012, 13:134.
18. Chakkour M, Hammoud Z, Farhat S, El Roz A, Ezzeddine Z, Ghssein G. Overview of Proteus mirabilis pathogenicity and virulence. Insights into the role of metals. Frontiers in Microbiology, 2024, 15, 1383618.
19. Hadi P, Rampal S, Neela VK, Cheema MS, Sarawan Singh SS, Kee Tan E, Sinniah A. Distribution of causative microorganisms in diabetic foot infections: A ten-year retrospective study in a tertiary care hospital in central Malaysia. Antibiotics, 2023, 12(4), 687.
20. Ramatla T, Mileng K, Ndou R, Mphuti N, Syakalima M, Lekota KE, Thekisoe OM. Molecular detection of integrons, colistin and β-lactamase resistant genes in Salmonella enterica serovars enteritidis and typhimurium isolated from chickens and rats inhabiting poultry farms. Microorganisms, 2022, 10 (2), 313.
21. Kot B, Grużewska A, Szweda P, Wicha J, Parulska U. Antibiotic resistance of uropathogens isolated from patients hospitalized in district hospital in central Poland in 2020. Antibiotics, 2021, 10(4), 447.

22. Al-Atabi WA. Study the effect of some biofilm inhibitors For Proteus mirabilis isolated from burns and wounds. M.Sc. Thesis, College of Education for Pure Sciences / Ibn Al-Haytham - Baghdad University, 2020.
23. Liu L, Dong Z, Ai S, Chen S, Dong M, Li Q, Peng G. Virulence-related factors and antimicrobial resistance in Proteus mirabilis isolated from domestic and stray dogs. Front. Microbiol. 2023, 14, 1141418.
24. Shaaban M, Abd El-Rahman OA, Al-Qaidi B, Ashour HM. Antimicrobial and antibiofilm activities of probiotic Lactobacilli on antibiotic-resistant Proteus mirabilis. Microorganisms, 2020, 8(6), 960.
25. Al-Zaidi OS, Abd Alhady Zwein L. Effect of tannic acid on urease and protease produced from Proteus mirabilis. J. Biotech. Res.Cen, 2019, 13(1), 82-86.
26. Mirzaei A, Habibi M, Bouzari S, et al. Characterization of antibiotic- susceptibility patterns, virulence factor profiles and clonal relatedness in Proteus mirabilis isolates from patients with urinary tract infection in Iran. Inf. Dr. Resist; 2019, 12:3967–79.
27. Scavone P, Iribarnegaray V, González MJ, Navarro N, Caneles Huerta N, Jara-Wilde J, Zunino P. Role of Proteus mirabilis flagella in biofilm formation. Revista Argentina de Microbiol, 2023, 55(3), 226-234.
28. Dincer S, Uslu FM, Delik A. Antibiotic resistance in biofilm. Bacterial biofilms, 2020, 10, 9.
29. Umpierreze A, Scavone P, Romanin D, Alejandre J, Chabalgoity J, Rumbo M, Zunino P. Innate immune responses to *Proteus mirabilis* flagellin in the urinary tract. 2013, 1-9.
30. AL-Fatlawi MR, Zwain OM, Alquraishi ZH. Role of Integrons 1 and 2 in Proteus spp. and Evaluation of Some Antibiotic's Resistance Genes. Egy. Acad. J. Biol. Sci. C, Phys. Molec. Biol, 2023, 15(2), 367-377.