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College of Science
Biology department

Molecular study of antibiotic resistance genes of Clinical isolates of *Proteus mirabilis*

A Thesis

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By

Israa Faize Ahmed Alobaidy
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Supervised By

Assist. Prof.
Dr. Iman Abbas Ali

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Summery

The total 250 clinical samples were gathered from various sources (urine, burns, wounds, diabetic foot, and vaginal swabs) from patients with different infections from Baquba Teaching Hospital, Al-Batool Teaching Hospital, and AlKhalis hospital in Diyala province during the period extended from August 2023 to January 2024 to be isolated, identified and confirmed for the presence of *Proteus mirabilis* bacteria.

The results demonstrated that 75 (30%) of the isolates were *P. mirabilis* distributed according to the sources from the highest to the lowest numbers and percentages as follows: urine, vaginal swabs, burns, diabetic foot, and wounds: 35 (46.7%), 12 (16%), 10 (13.3%), 10 (13.3%) and 8 (10.7%) respectively depending on the traditional methods of using specific differential media, microscopic characteristics, biochemical tests. All 75 *P. mirabilis* isolates showed positive results for swarming, KIA, catalase, methyl red and citrate, while they showed negative indole and oxidase enzyme results.

Seventy-five *P. mirabilis* isolates were examined to detect multidrug and extensive drug resistant against 15 antibiotics, It was found that the more effective antibiotic against isolates was Norfloxacin 100%, where all isolates were sensitive to it, followed by Levofloxacin 93% and Ofloxacin 87%. On the other hand, the less effective antibiotics were Amikacin and Oxacillin when they were resisted by all isolates 100%, followed by Amoxicillin 97%. In addition, these isolates showed variable resistance rates to the Aztreonam 47%, Imipenem 17%, Cefoxitin 3%, Ceftazidem 60%, Ciprofloxacin 3%, Nalidixic acid 33%, Gentamicin 50%, Kanamycin 63%, Amoxicillin-clavulanic acid 27%. It has been found that 73 (97%) of all isolates were found to be MDR, while only 2 isolate was tended to be XDR (3%) while no PDR was detected.

The present results showed that all isolates of *P. mirabilis* were phenotypically positive 100% for urease and biofilm formation in different degrees. The study also demonstrated that (37%) and (73%) of *P. mirabilis* producing β-lactamase and ESβ-lactamase respectively.

Regarding the prevalence of resistance genes, the present findings revealed that the 12 resistant isolates were subjected to Uniplex PCR to amplify resistance encoding these genes. It has been found that the prevalence of *16sRNA*, *aac(6')-Ib*, *blaTEM*, *Intl1*, *Intl2* as (100%), (33.33%), (25%), (75%) and (50%) rates respectively. One isolate, number (2) isolated from diabetic foot infection was identified as standard strain has been placed in the National Center for Biotechnology Information (*NCBI) with the accession number PP593933.1, LC810419.1, LC810416.1, LC810417.1, LC810418.1 for the genes 16S+ ribosomal RNA gene, *blaTEM*-1, *aac(6')-Ib, IntI1, IntI2,* respectively.

A total of twelve samples (assigned A1 to A12) were amplified from the *16S rRNA* locus. The differences of these ribosomal sequences showed that 99% homology between the sequenced sample of A1 – A12 and *P. mirabilis* reference target sequences (GenBank acc. OL629230.1) and A total of four samples (assigned B1 to B4) were amplified from *IntI* locus. The sequencing responses specified the accurate identity after running NCBI blastn for these PCR amplicons. The NCBI BLASTn engine showed 100% homology between the sequenced sample of B1 – B4. *P. mirabilis* reference target sequences (GenBank acc. KP66515.1).

A total of four samples (assigned C1 to C4) were amplified from *IntI1* locus. The NCBI BLASTn engine showed 99% homology among the sequenced sample of C1 – C4 and *P. mirabilis* reference target sequences (GenBank acc. CP138492.1). A total of four samples (assigned D1 to D4) were amplified from *aacIb* locus. The NCBI BLASTn engine showed that 99% homology between the sequenced sample of C1 – C4 and *P. mirabilis*

reference target sequences (GenBank acc. CP015347.1). A total of three samples (assigned E1 to E3) were amplified from *blaTEM* locus. The NCBI BLASTn engine showed 100% homology among the sequenced sample of C1 – C4 and reference target sequences (GenBank acc. CP148143.1).

Thorough phylogenetic tree of MDR of *P. mirabilis* was created in the current study based on nucleic acid sequences within amplified PCR products of the *16S rRNA, IntI1, IntI2, aacIb, and blaTEM* amplicons of *P. mirabilis*. By comparing the genetic sequences obtained from *P. mirabilis* isolates from various sources, variable insights into the diversity and relatedness of strains are obtained. The phylogenetic analysis allows for the classification of *P. mirabilis* isolates into several distinct clades, which can provide information about the spread and transmission of specific strains.

Chapter one

Introduction

1. Introduction

Proteus mirabilis is a Gram-negative, facultative anaerobic bacteria from the Morganellaceae family. It is found throughout the natural world and is a component of the gut flora of both humans as well as animals. Though, it is blamed for numerous nosocomial and community-acquired epidemic around the world, including respiratory tract infections and urinary, diabetic foot ulcers, and a variety of other illnesses (Armbruster *et al*., 2018). *P. mirabilis* causes widespread infection because it has various virulence factors such as adhesion, toxins, flagella, and enzyme production (such as urase), biofilm, as well as an extensively antibiotic-resistant phenotype (Aghapour *et al*., 2019).

These virulence factors each play an important function in UTIs. These elements are related to the connection between bacteria and surfaces, attack, destruction to tissues of the host, and escape from the immune system of the host (Maszewska *et al*., 2021). Historically, a majority of *P. mirabilis* isolates were sensitive to common antibiotic classes. Previeus studies shows the resistance of the antibiotic is rising among *P. mirabilis* isolates from various regions. (Lv *et al*., 2022). The occurrence of multidrug-resistant (MDR) strains of *P. mirabilis* in some contexts could be rather high due to extended-spectrum beta-lactamase (ESBL), MDR could be caused by genetic changes in resistance genes, or resistance genes can be acquired through horizontal movement (Sfaciotte *et al.*, 2021).

Clinicians may have limited therapy options due to the spread of multidrug resistant (MDR) and extensive drug resistance (XDR), and pandrug resistant microorganisms (Algammal *et al*., 2021). As a result, it is regarded as a primary driver of the spread of drug resistance features across several bacterial pathogens, particularly within the Enterobacteriaceae family (Facciolà *et al*., 2022). In nosocomial infections, the World Health

Organization has focused on this multidrug resistance pathogen, classifying it as a medically relevant nosocomial and community-acquired disease. Antibiotic using badly, has resulted in more advanced stages of drug resistance and the proliferation of many resistance genes among clinical *P. mirabilis* isolates; also, *P. mirabilis* is intrinsically resistant to tigecycline, tetracycline, as well as polymyxin (Sanches *et al*., 2023).

β-lactam antibiotics, such as cephalosporins, penicillin, and carbapenems, are the first line of treatment for *P. mirabilis* infections. Enzymatic hydrolysis of β-lactam antibiotics is a major resistance mechanism (Mirzaei *et al*., 2021). Choosing the right antibiotic requires understanding the functional as well as structural classification of β-lactamases (de Oliveira *et al*., 2021). Carbapenem resistance is slightly rare, however it is growing over time. Lately, increasing resistance to quinolones and aminoglycosides has been observed worldwide (Salama *et al*., 2021).

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 (Obadire *et al*., 2022). 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 (Hu *et al*., 2020). These genes allow *P. mirabilis* to withstand a variety of antibiotics, including beta-lactams, quinolones, chloramphenicol trimethoprim, aminoglycosides, and rifampicin (Firmo *et al*., 2020).

This study was performed to evaluate and identify a sequence of the resistant of antibiotic genes (*blaTEM*, *aac(6')-Ib*, *IntI2*, *IntI1*), that examines genes with resistance and contains a mobile genetic factor in multidrug resistant *P. mirabilis* in clinical samples.

The aims of current study:

Detect *P. mirabilis* bacteria with multiple resistance to antibiotics by bacteriological and biochemical methods and to determine some virulence factors by phenotype methods. The current study also aimed to detection of some resistance genes genotypically by conventional PCR as well as identify and characterize the phylogenetic positioning of *P. mirabilis* that are amplified from five different loci (*16S rRNA*, *IntI1*, *IntI2*, *aacIb*, and *blaTEM*) from the targeted bacterial isolates.

These aims established by:

1. Isolation and identification of *P. mirabilis* from several clinical sources by morphological and biochemical methods.
2. Studying the sensitivity of *P. mirabilis* against beta lactam, aminoglycoside, quinolone antibiotics.
3. Detection some virulence factors phenotypically using microbiological methods.
4. Molecular detection of some Antibiotic Resistance Genes (*IntI1*, *IntI2*, *aacIb*, and *blaTEM*) by using singlplex, PCR and gene sequencing methods.

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دراسة جزيئية لجينات المقاومة للمضادات الحيوية للعزلات السريرية لبكتريا *Proteus mirabilis*

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بإشراف

الأستاذ المساعد

د. ايمان عباس علي

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