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Molecular Identification Of Virulence Factors genes of *proteus mirabilis* Isolated From Urinary Tract Infection

A Thesis

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Introduction

Proteus belongs to the Enterobacteriaceae of Gram-negative bacteria that are facultatively anaerobic. Gustav Hauser was the first to discover it in 1885. The willingness of its species to undergo morphological changes inspired the name of this genus, *Proteus* species are motile and have four to ten peritrichous flagella. (Ryan and Ray, 2010).

Proteus is an opportunistic pathogen that can cause serious invasive diseases in people who are chronically ill, elderly, or pregnant. It is a major cause of infections acquired in hospitals (Hamilton *et al*., 2018). *Protues mirabilis* can cause urinary tract infections, respiratory tract and wound infections, burns, and digestive tract infections, among other things (Smelov *et al*., 2016).

Protues mirabilis has many virulence factors like flagellum, capsules, fimbria and enzymes like (deaminase amino acid, urease and proteases), toxins such as endotoxins ,hemolysins and proteus toxic agglutinin (Pta), that detected using molecular techniques which explains the difficulty in achieving clinical therapy effectiveness (Cestari *et al*., 2013).

Protues mirabilis secretes a variety of cell-associated factors, including swarming, fimbriae, urease, proteolytic activity, and the development of hemagglutinins and hemolysins, iron acquisition systems, protease, and Lipopolysaccharides (LPS). This pathogen has evolved some pathogenic factors that allow it to colonize, survive, and develop in its host.

Protues mirabilis is also noted for its ability to shape biofilms that colonize routes and thus avoid antibiotic treatment. *Proteus* species are

the third most common cause of urinary tract infections (Flores-Mireles *et al*., 2015b).

A urinary tract infection (UTI) occurs when a pathogen enters the urinary tract system and multiplies to over 10^5 colonies per milliliter of urine, UTI is the second most common cause of infectious disease, affecting over 150 million people worldwide (Mann *et al*., 2017).

Protues mirabilis has been showing an increase in resistance to many antimicrobial agents in recent years (Gajdács and Urbán, 2019).

Antibiotic resistance has resulted in not only improvements in antimicrobial therapies, but also poor prognoses and a rise in hospitalized patient mortality (Giamarellos-Bourboulis *et al*., 2006).

Protues mirabilis has gained resistance to many antibiotic types, making treatment more difficult. Resistance to -lactams (both penicillins and cephalosporins), fluoroquinolones, nitrofurantoin, fosfomycin, aminoglycosides, tetracyclines and sulfonamides, in addition to the previously mentioned resistance to sulfamethoxazole and trimethoprim, has been identified (Schaffer and Pearson, 2017).

Thus, there are many virulence genes that assist survival of *P. mirabilis* within the urinary system such as urease, hemolysin, fimbriae, and flagella. However, *P. mirabilis* strains differ in the range and expression levels of virulence genes that can affect growth of bacteria and persistence within the urinary tract Abbas et al.,(2015).

A number of studie s have investigated the virulence characteristics of *P. mirabilis* and mechanisms involved in pathogenesis of UTI to identify the range of *P. mirabilis* virulence genes and their prevalence among *P. mirabilis* isolates(Hussein *et al* 2020) .In the present study, *P. mirabilis* isolates involving in human UTI are characteriz to identify

virulence gene markers in an effort to explore strategies involved in *P. mirabilis* pathogenesis and antibiotics susceptibility.

The aim of study:

This study was aimed to detect some virulence factors of uropathogenic *P. mirabilis* using multiplex PCR method. To achieve this goal, the steps of this study are:

1- Isolation and identification of *P. mirabilis* from patients with urinary tract infections.

2- Study the susceptibility of *P. mirabilis* toward certain traditional antibiotics.

3- Phenotypic detection of some virulence factors of *P. mirabilis* using cultural methods.

4- Molecular detection of some virulence factors using singlplex, multiplex PCR and gene sequencing methods.

Summary

This study was aimed to detect some virulence factors of *Proteus mirabilis* isolated from patients with urinary tract infection using molecular methods. 300 urine specimens were collected from patients who attended (Al-Khalis general hospital, Al- Batool Teaching Hospital and Baquba Teaching Hospital) for a period extended from the 5th of August 2020 to 25th of January 2021. The diagnosis was done by consultant urologist. The age range of patients was from 2 to 56 years old. The isolation and identification of *P. mirabilis* was achieved by conventional methods including (Bacteriology, biochemical and Vitek kit) and molecular methods including (single-plex PCR of *ZapA* gene, *Urec* gene, Mrp gene, *FlaA* gene and *Esp* gene, multi-plex PCR *ZapA* gene and *FlaA* gene of and gene sequencing of *ZapA*). The antimicrobial susceptibility to 18 antibacterial was done using Kirby-Bauer disk diffusion method. Furthermore, The MIC for Cifipime and Meropenem was determined by microtiterplate .

According to the conventional detection methods, 25 isolates were diagnosed with 8.3% *protues mirabilis* bacteria from 300 samples. The highest isolation rate was found to be among those (20-29) years old patients 36.0% . Female 64.0%, rural residence 60.0%, acidic urine 76.0%, urine samples with10 pus cells/HPF, and urine samples with 10 RBCs/HPF. Regarding the virulence factors; all isolates 100% were positive for hemolysin, urease, siderophore, colony-factor antigen I, II, and III. Additionally, 11(44.0%) are strong and 11(44.0%) are moderate biofilm former, while only 3(12.0%) were non-biofilm formers. According to the susceptibility of studied antibiotics, the results showed that 17 out of 25 (68.0%) of *P. mirabilis* isolates were multi-drug resistant (MDR), while the remaining 8 32.0% were extended drug-resistant (XDR) isolates.