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العزل و التوصيف الجزيئي ل *Proteus Mirabilis* من التهابات المجاري البولية للانسان و الاغنام في محافظة ديالى

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Isolation and Molecular Characterization of *Proteus Mirabilis* from Urinary Tract Infections of Human and Sheep in Diyala Province

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Chapter One

Introduction

Introduction

Urinary tract infections (UTIs) are one of the most common infectious diseases, and nearly 10% of people will experience a UTI during their lifetime.(Safar Farajnia, Alikhani et al. 2009) .

Although several different microorganisms can cause UTIs, including fungi and viruses, bacteria are the major causative organisms ,they are responsible for more than 95% of UTI cases (Bonadio, M. et al. 2001)

Urinary Tract Infection defines a condition in which the urinary tract is infected with the pathogen causing inflammation, major pathogenic bacteria causing urinarytract infection is gram negative bacteria which belongs to the *Enterobacteriaceae* family(Al-Jeboury 2005), so *Proteus* is considered as the main causative agent of urinary tract infection after *Escherichia coli* , especially *Proteus mirabilis* (Ramakrishnan, K. et al. 2005).

Proteus species known as opportunistic bacteria that are gram-negative, belong to the family Enterobacteriaceae, They are widely distributed in natural environment and as microbiota in human and animal intestines (Drzewiecka, 2016).

In suitable conditions, they can cause infections in wound, skin and urinary tract infections (UTIs) in animals and humans (Kim BN, Kim NJ et al. 2003, Xiang Yu, Agnieszka Torzewska et al. 2017, Schaffer JN and MM. 2015), and can cause rheumatoid arthritis (Xiang Yu, Agnieszka Torzewska et al. 2017).

Proteus contains seven species that named *P. mirabilis*, *P. penneri*, *P. vulgaris*, *P. myxofaciens*, *P. hauseri*, *P. terrae*, and *P. cibarius* and three unnamed *Proteus* genomo species, 4, 5, and 6 (O'Hara CM, Brenner FW et al. 2000, Behrendt U, Augustin J et al. 2015, Hyun DW, Jung MJ et al. 2016).

P. mirabilis, *P. penneri*, and *P. vulgaris* among other species, are the most common pathogens, and isolates of *P. mirabilis* cause UTIs with the highest frequency. *P. myxofaciens*, *P. terrae*, and *P. cibarius* have no pathogenicity for humans (Rozalski A, Sidorczyk Z et al. 1997, Jacobsen SM, Stickler DJ et al. 2008, Behrendt U, Augustin J et al. 2015).

The pathogenesis of these bacteria is associated with possessing many virulence factors which include the pili (Fimbria) ,Flagella ,Urease , Protease , Heamolysin ,and multi-sugars adipose (lipopolysacchride) and Endotoxin(Sosa, V.Schlapp et al. 2006)

P. mirabilis is capable of causing symptomatic infections of the urinary tract including cystitis and pyelonephritis and it is present in cases of asymptomatic bacteriuria, particularly in the elderly and patients with type 2 diabetes (Papazafiropoulou A, Daniil I et al. 2010, Matthews SJ and JW. 2011).

These infections can also cause bacteremia and progress to potentially life-threatening urosepsis. Additionally, *P. mirabilis* infections can cause the formation of urinary stones (urolithiasis).(Alazzwi and Raheem 2011)

About 1-10% of all UTI can be caused by *P. mirabilis*, varying with the geographic location of the study, the types of samples collected, and the characteristics of the patients examined(N., Schaffer et al. 2015)

Therefore the aim of this study is:

[1] Isolation and identification of *P. mirabilis* from urine of human and sheep infected with UTIs.

[2] Studying the frequency of *P. mirabilis* associated UTI in human and sheep

[3] Studying the ability of biofilm formation by *P.mirabilis* in vitro

[4] Molecular detection of *ZapA* and *Hpma* as gene coding for virulence factors (extracellular metalloprotease, hemolysin) and their role in biofilm formation and UTI

[5] Detection of the resistance for anti-microbial therapies in vitro, and study the inhibition of biofilm by probiotics.

Abstract

Background and Objectives:

Proteus mirabilis is a predominant bacterial etiology of urinary tract infections (UTIs). The aim of this study is isolation and identification of *P. mirabilis* causing UTI in human and sheep, molecular characterization of ZapA Metalloprotease and HpmA hemolysin genes; Study the anti-biofilm activity of cell free supernatants of *Bacillus subtilis* KATMIRA1933 and Bacillus amyloliquefacience B-1895 against biofilm-associated *P. mirabilis*

Method:

This study was performed starting from October 2017 to April 2018. Mid-stream urine samples were collected from 250 human and 190 sheep and inoculated into blood and MacConkey agars for detection of *P. mirabilis*. Antibiotic susceptibility and multidrug resistance of *P. mirabilis* were evaluated .Molecular characterization of ZapA Metalloprotease and HpmA hemolysin genes by qRT-PCR were identified in this work. Furthermore, the anti-biofilm activity of cell free supernatants of Bacillus subtilis KATMIRA1933 and Bacillus amyloliquefacience B-1895 were evaluated against biofilm-associated P. mirabilis

Results

The mean age of patients with UTI was (27.88 ± 11.680) years old. A total of 60/250, (24%) human samples revealed positive P. mirabilis. The age group (17-23 years); 33.33 % was frequently infected with P. mirabilis while (3-9 years), (10-16) years were less frequently exposed, each with 4%. A total of (66. 67%) of patients with P. mirabilis have secondary education, while 1.67% of patients have primary education. In addition, 93.33% of patients drinking filtrated water. According to the residency, 63.33% of positive cases were living in Baqubah-al takia while (3.34%) from Al-Khalis and Khan keen. patients with UTI-associated *P. mirabilis* suffered from flank pain (63.33%) and fever (45%), hematuria (43.33%) and dysuria (13.33%).

The mean age of sheep presented with UTI was (8.93 ± 2.840) months. A total of 30/190, (15.78%) samples showed *P.mirabilis*. A total of

(43.33%) of UTI-associated *P. mirabilis* cases was determined in (10-12 months) age group. All positive cases were drinking river water and tap water (mixed). A total of (80 %) of *P. mirabilis*-UTI positive cases was from Khan Bani-Saad while (3.33%) was from Al-Mufraq. A total (50 %) of Sheep with positive *P. mirabilis*-UTI, suffered from hematuria and dysuria was reported in (23.33%) only.

P. mirabilis that isolated from human and sheep with UTI were resistant to ampicillin (10µg), amoxicillin-clavulanic acid (10µg-20µg), most of them were resistant to ceftriaxone (30µg), cefotaxime (30µg), and sensitive for amikacin (30µg). Bacteria shows multidrug resistant for Ampicillin, Amoxicillin-Clavulanic acid ,Ceftriaxone, Cefotaxime, Cefixime ,Ciprofloxacin ,Trimethoprim–sulfamethoxazole , Nitofurantoin and Nalidixic acid in percentage of 40-90% .

The mean number of *HpmA* copies that was detected in qRT-PCR was (5930.2900± 7113.26530 copy/µl), and the mean number of *ZapA* copies was (6250.2270 ± 8952.47838 copy/µl).

A 25% and 50% of cell free supernatant of *Bacillus subtilis* KATMIRA1933 (BSK1933) were effectively preventing 70% and 84% respectively, of biofilm –associated *P. mirabilis* isolated from patients with UTI. Moreover, 64% and 80% of biofilm –associated *P. mirabilis* isolated from patients with UTI were effectively preventing when 25% and 50% of CFS of *Bacillus amyloliquefacience* B-1895(BAB-1893) were used. For the biofilm-associated *P. mirabilis* isolated from UTI infected sheep, 41% and 47% of biofilm inhibited when CFS of BSK1933 was used while 28% and 60% of biofilm was prevented when CFS of BAB-1893 at 25% and 50%, respectively were used.

Conclusions

P. mirabilis associated UTIs is distributed among human and sheep especially at younger age groups. Life style factors increasing the possibility *P. mirabilis* associated UTIs. *HpmA* and *ZapA* genes play a vital role pathogenesis and severity of *P. mirabilis* infection. BSK1933 and BAB-

1893 represent a promising alternative therapy for UTI to counteract the multidrug resistant problem in *P. mirabilis*.