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Gene Detection of Some Virulence Factors In *Klebsiella Pneumoniae* Isolated From UTI

A Thesis

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بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

﴿ يُؤۡتِي ٱلۡحِكۡمَةَ مَن يَشَآءُۚ وَمَن يُؤۡتَ ٱلۡحِكۡمَةَ فَقَدۡ أُوتِيَ خَيۡرٗا كَثِيرٗاۗ وَمَا يَذَّكَّرُ إِلَّآ أُوْلُواْ ٱلۡأَلۡبَٰبِ ٢٦٩ ﴾

صَدَقَ ٱللَّهُ ٱلۡعَظِيمُ [1]

[1] البقرة: 269.

Dedication

To My Parents

For toughing me to trust in Allah, all

the unconditional love, guidance, raised me to be the person I am today, supporting and encouraging me to believe in myself

My Husband

For his remarkable patience and unwavering love and support over the many years we have been together

My Brothers and Sister

For have been great sources of motivation, love and inspiration for me

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Sara

Supervisor Certification

We, Certify that this thesis entitled **(Gene Detection of Some Virulence Factors In *Klebsiella Pneumoniae* Isolated From UTI)** has been conducted under our supervision at College of Medicine, University of Diyala, as a partial requirements for the Master Degree in Medical Microbiology.

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Abstract

A total of 160 clinical samples were collected from two hospitals in Diyala from patients in both sexs, suffering from urinary tract infection of age range between(≤ 10-50) years in the period between (September / 2019 –December / 2019). Out of 160 isolates, 24 identified as *Klebsiella pneumoniae* depending on cultural, biochemical characteristics, and molecular methods. The remaining bacterial isolates from urine samples were identified as *Escherichia coli (*45) , Pseudomonas(23), Proteus(27 and Citrobactar(16) isolates.

Regarding to the patients gender, it was found that females had a tendency to get infection more than the males when 20 (83.3%) of patients were females and 4 (16.7 %) of them are males. Moreover, the age group (30-39) were most subjected to the infection of *K. Pneumoniae.*

The antibiotic susceptibility test showed that all isolates were Multidrug Resistance (MDR) , were high resistant to Ciprofloxacin 24 (100%),Cefotaxime 24(100%) ,Piperacillin 17(70%), Nalidixic acid 12(50%), Tetracycilln 10 (41%), Nitrofurantion8(33%), Chloramphenicol 7(29%) Ampicilln-sulbactam7(29%), Tobromycin4(16%), and Impenem 2(8%).

The organism were identified by using molecular methods, including the DNA extraction and checking with PCR using identified genes, including *16srRNA*,and some virulence factor gene such as *rmpA* and *iroN gene* .

The DNA sequencing was done to identify the strains of *Klebsiella pneumoniae* that cause UTI in Iraq especially in Diyala city, the results showed that there are two strains responsible to the UTI in this city, *Klebsiella pneumoniae* strains D16KP0042 and KPWIQ25. The results of DNA sequencing analysis of *16SrRNA* gene from k1 to k24 isolates revealed that

k.pneumoniae were (100%).and iro N gene revealed only in 3 isolates(12.5%),while rmp A gene not observed in any isolates.

The prevalence of biofilm producer bacterial isolates was 22(91%) that 7(31%) produced strong, 6(27%) produced moderate and 9(40%) produced weakly biofilm.

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List of Abbreviations

Abbreviate	Key
AMP	Antimicrobial Peptides
$BaCl_2$	Barium chloride
C3b	Complement protein
CFU	Colony forming units
CPS	Polysaccharides of Capsule
CRBSIs	Catheter-relatedbloodstrea infections
CRKP	Carbapenem-resistant *Klebsiella pneumonia*
D.W.	Distilled water
ESBL	Extended spectrum beta-lactamases
HAP	Hospital acquired pnemoniae
ICU	Intensive care unit
K. pneumoniae	*Klebsiella pneumoniae*
LPS	Lipoplysaccharide
MDR	Multidrug-resistance
PBS	Phosphate BufferSaline
PCR	Polymerase Chain Reaction
RmpA	Regulator of phenotype gene A
rRNA	Ribosomal Ribonucleic Acid
SOD	Superoxide dismutase
UTI	Urinary tract infection
VP1-VP2	Vogas-Proskauer

Chapter One

Introduction

Introduction.

Klebsiella pneumoniae is a Gram-negative rod in the Enterobacteriaceae family. The bacterium is found indigenously in soil and waters, but also on mucosal surfaces in mammals, including humans (Podschun and Ullmann, 1998).

Klebsiella pneumoniae is responsible for many community –onset and nosocomial infections. The increasingly high level of antimicrobial drug resistance prevalence is an exaggerated problem, especially for healthcare providers. These bacterium can confer resistance to the majority of antibiotics by applying vast amounts of resistance mechanisms, leading to high mortality and morbidity rates. The dominant antibiotics used for treating this bacterium infections today are the β-lactam antibiotics, which inhibit transpeptidases participating in bacterial cell wall synthesis. Beta-lactam antibiotics can be deactivated by β-lactamase enzymes (Paterson and Bonomo, 2005).

Antibiotic resistance represents one of most significant healthcare problems. The loss of effective antibiotics would weaken the ability to fight infectious diseases and treat the complications for patients with renal dialysis, cancer patients with chemotherapy, and organ transplantation surgery (Organization, 2014).

The choice of an adequate antibiotic regimen for the treatment of infections due to Multidrug resistant (MDR) strains of *K. pneumoniae* is a challenge for physicians for the patients which develop serious infections which is from patients hospitalized considering that patients in many cases develop serious infections and complications , these infections affect not only critically ill patients hospitalized in intensive care unit (ICU) but also patients from other wards with multiple comorbidities(Bassetti et al., 2017) . The important and recognizable virulence factors and quorum sensing (QS) are involved in higher degree of infection in *Klebsiella pneumoniae*. QS is responsible for change in community of bacteria for their mobility alteration, different enzymes production which may help them to

invade and estabilish in tissues of host, toxic compound production, Slime and capsule components production and formation of aggregates in the form of flocks and biofilm (Papenfort and Bassler, 2016).

Biofilm formation with the help of QS can be recognized as intrinsic antibiotic resistance factor in this type of aggregates makes impermeability in a structure and stop antibiotic to interact with a bacteria and increased the antibiotic resistance (Vuotto et al., 2014). Therefore, it is essential to study the relationship between antibiotic resistance and biofilm formation in *K. pneumoniae*.

1-2 Aim of study :

1. To identify *k. pneumoniae* isolated from urinary tract infection patients using 16 S gene sequencing.
2. To find the antibiotic sensitivity of *K. pneumoniae* strains.
3. To investigated the rate of occurrence of biofilm in *k. pneumoniae* isolated from UTI.
4. To identify the correlation with different parameters such as gender, age, and chronic disease