Bacteriological and Molecular Study of *Klebsiella Pneumoniae* Isolated from Patients with Urinary Tract Infections from Several Hospitals in Baghdad

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Abstract

This study obtained 20 isolates of *Klebsiella pneumoniae* from out of 50 isolates collected from patients with urinary tract infection; all isoates were diagnosed and then the sensitivity test for isolates was performed using the discs method (Kirby-Bauer) to determine the resistance of *K.pneumoniae* to 10 antibiotics. The results detected that the highest resistance was against Ampicillin, Cefotaxim, and Piperacillin by 100% for each of them, while the rest of the antibiotics had a resistance percentage of 80% for Trimethoprim, 54% for Gentamicin and 65% for Azithromicin, however the lowest resistance was found against Imipenem, Chloramphenicol and Ofloxacin by 4%, 12. % and 35% respectively. The genomic bacterial DNA of studied isolates was extracted and the genotyped was performed identify the genetic relationships between the bacterial isolates using ERIC-PCR method. The results of the study showed the presence of 12 genotypes and also the results revealed the presence of two clones, each of which contains similar genotypes, while the rest of the isolates contain different genotypes.

Keywords: *Klibsiella pneumoniae, antibiotic resistance, ERIC-PCR.*

Introduction

Klebsiella pneumoniae is one of the most important genera of the Enterobacteraceae family. It is characterized by being Gram-negative bacilli, non-motile, and lactose fermented, non spore forming, appear under the microscope are made up of thick edges, as the edges are curved outward and have rounded ends and have a capsule that increases its pathogenicity (Braun *et al*., 2004; Bensen 2001; Sambrook *et al*., 2001). It was called by this name in the nineteenth century in relative to its discoverer Edwin Klebs, a German-born scientist, in 1834 (Brise *et al*. 2005).

K.pneumoniae is one of the causative agents of opportunistic diseases in humans, including respiratory tract infection, Burns inflammation, wounds inflammation, septicemia, diarrhea, and liver abcesses (Zhang *et al*., 2018) .

Urine tract infection is one of the most common diseases caused by *K. pneumoniae* after *Escherichia coli*. Moreover, it is become more dangerous for people who suffer from Diabetics, alcoholics, pulmonary deficiency, immune suppressive, and hospitalized patients especially in intensive care units, thus the accurate and rapid diagnosis is required to prevent the infection in hospitals (Li *et al*, 2012; Chiu *et al*., 2013; Guo *et al.* 2016).

K. pneumoniae bacteria possess many virulence factors including production of polysaccharide, capsule, serum resistance, production of iron siderophore, production of enterotoxin and urea and thus this bacteria with high virulent in addition to developing its resistance to many antibiotics (Navan-venzia *et al*., 2017; Dubey *et al.,* 2013).

The major reasons for *K. pneumoniae* resistance to antibiotics including beta-lactam antibiotics is due to producing the broad-spectrum of beta-lactamase (ESBL) which is one of the most important problems of increased infection in hospitals, or through the changing of the permeability barrier or in the target site

represented by penicillin binding protein, or alteration of outer membrane protein (Aghamohammad *et al*., 2018). Additionally, *K. pneumoniae* havine many Efflux Pumps that expel the antibiotic to the outside. Beta-lactam antibiotics include a group of antibiotics including penicillins, cephalosporins, carbenems and monobactam (Livermore, 2012; Sachse *et al*., 2012, Levinson., 2016).

Trimethoprim inhibits dihydro pterotese Synthetase (DHPS) and dihydro folate reductase (DHFR), which are involved in the synthesis of bacterial DNA, and *K. pneumoniae* showed high resistance to Trimethoprim. The reason for this resistance is via modulating the target site that bind with the enzymes, and also having encoded genes carrying on conjugate plasmids that may be acquired or given to other bacteria present in the same culture medium, as well as containing a flow mechanism that changes the membrane permeability (Shin *et al*., 2015).

Moreover, *K. pneumoniae* possesses multiple mechanisms to resist aminoglycoside; these mechanisms including its production of the three modulating enzymes: Acetyl transferase, Phospho transferase and Adenal transferase (Serio *et al*., 2017). Additionally, *K. pneumoniae* have several mechanisms of resistance to antibiotics including Quinolones antibiotics such as Ciprofloxacin, Norofloxacin, Levofloxacin and Ofloxacin. These mechanisms invovlved the modulation of the target site that bind with the antibiotics by triggering chromosomal genetic mutations in the genes encoding for DNA grease or Topoisase resulting in producton of different enzyme that is resistant to these antibiotics. *K. pneumoniae* also have OqxAB and QepA efflux Pumps on the outer membrane of the bacteria that serve to expel the antibiotic to the outside and prevent the permeability of it (Jacoby, 2017).

Genotyping method are important in finding the genetic affinity between bacterila isolates and also in the classification of bacteria, identification the sources of infection and characterization of the most pathogenic strain. There are several method of genotyping including Enterobacterial Repetitive Intergenic consensus method which is easy and rapid method that does not need long time and less expensive in comparison with other method (Goudarzi *et al*., 2011).

Aims of the study:

1. Isolation and diagnosis of *Klebsiella pneumoniae* from patients with urinary tract infections.
2. Determination the resistance and sensitivity of *Klebsiella pneumonia* to antibiotics.
3. Detection the genotyping and genetic relationships of *Klebsiella pneumoniae* using ERIC-PCR method.

Materials and Method

1. **Bacteria isolation:** 50 samples were collected from patients with urinary tract infections, male and female, from several hospitals in Baghdad (Central Child Hospital, Medical City, Al-Yarmouk Hospital, Child Protection Hospital) for the period from 1/10/2019 to 6/12/2019.
2. **Diagnosis of Isolates:** Samples were diagnosed using blood agar and MacConkey agar culture media. For final diagnosis of isolates, Vitek 2 system was used.
3. **The sensitivity of the bacterial isolates** was perfomed using the discs diffusion method (Kirby-Bauer) to determine the resistance of *K. pneumoniae* for 10 antibiotics included Ampicilin, Cefotaxime, Piperacillin, Gentamycin Sulfamethoxazole + Trimethoprime, Imipenem, Ciprofloxacin, Azithromycin, Ofloxacin and Chloramphenicol. The measurement of the inhibition zone diameter with around the antibiotic discs was perfomed and compared with the tables of international measurements (CLSI, 2017).
4. **DNA Extraction:** Genomic DNA extraction and Purification Kit was used to extract the bacterial DNA according to manufacturer instructions (Promega,USA).
5. The genotyping of ***K.pneumoniae*:** it was perfomed using PCR technique to detect the genotype of ERIC gene using the following primers:

   ERIC (F): 5 ′ - ATG TAA GCT CCT GGG GAT TCA C-3

   ERIC (R): 5 ′ - AAG TAA GTG ACT GGG GTG AGC G-3

The results of this test included the production of bands with variable size. The reaction mixture included 20 µL of GO Taq Green Master Mix10, 2 µL of DNA template, 2 µL of each primer and 6 µL of deionized (molecular) distilled water. The reaction conditions was programed according to the manufacturer instructions (Promga, USA) with some modifications as following (Mehr et al., 2017):

1. The initial denaturation with the single cycle at 94° C for 3-minute.
2. DNA amplification using one cycle at 94° C. In order to attach the primer with DNA templet, 35 cycles was used and each cycle included the following steps:

A- Anneling stage : one cycle at 48° C for 1 minute.

B- Extension stage: at 72° C for 2 minutes.

C- Final Extension stage: one cycle at 72° C for 5 minutes.

6. The reaction products were separated using agarose gel (Bio Basic INC, Canada) (2%) containing 5 µl of Eithidium bromide (Promega USA), and using DNA ladder (100-1500) base pair with a voltage difference of 100 volts for 80 minutes and imaged using UV light (Optima, Japan).

Results and Discussion

After performing the laboratory tests, 20 isolates of *Klibsiella pneumoniae* were obtained from a total of 50 samples collected from patients with urinary tract infections from several hospitals in Baghdad.

K.pneumoniae are among the most important causes of urinary tract infections, fees, burn, respiratory tract infections and bacteremia (Le *et al.*, 2012).

The results of this study revealed that the highest resistance and sensitivity of *K. pneumoniae* to the studied antibiotics was against Ampicillin by 100%, Cefotaxime by 100%, Piperacillin by 100% and for Trimethoprim by 80%, while the rest of the antibiotics, the resistance percentage was 54% for Gentamicin and 65% for Azithromicin. Furthermore, the lowest resistance was against Imipenem 4%, 12% for Chloramphenicol and 35% for Ofloxacin as shown in Figure (1).

The findings of this study are in agreement with Mehr *et al*. (2017) study that determined the resistance of *K. Pneumoniae* isolates which isolated from several hospitals in Korea; it was found that the resistance rates to Imipenem, Ampicillin and Azithromicin were at rates of (4%, 91%, 37%) respectively, as found in this study. Additionally, our results were consistent with Vasaikar *et al.,* 2017 observation that found the resistance of *K. pneumoniae* isolates isolated from several hospitals in South Africa was 29.7% for Ciprofloxacin, 51% for Gentamicin and 70.8 for Trimethoprim.

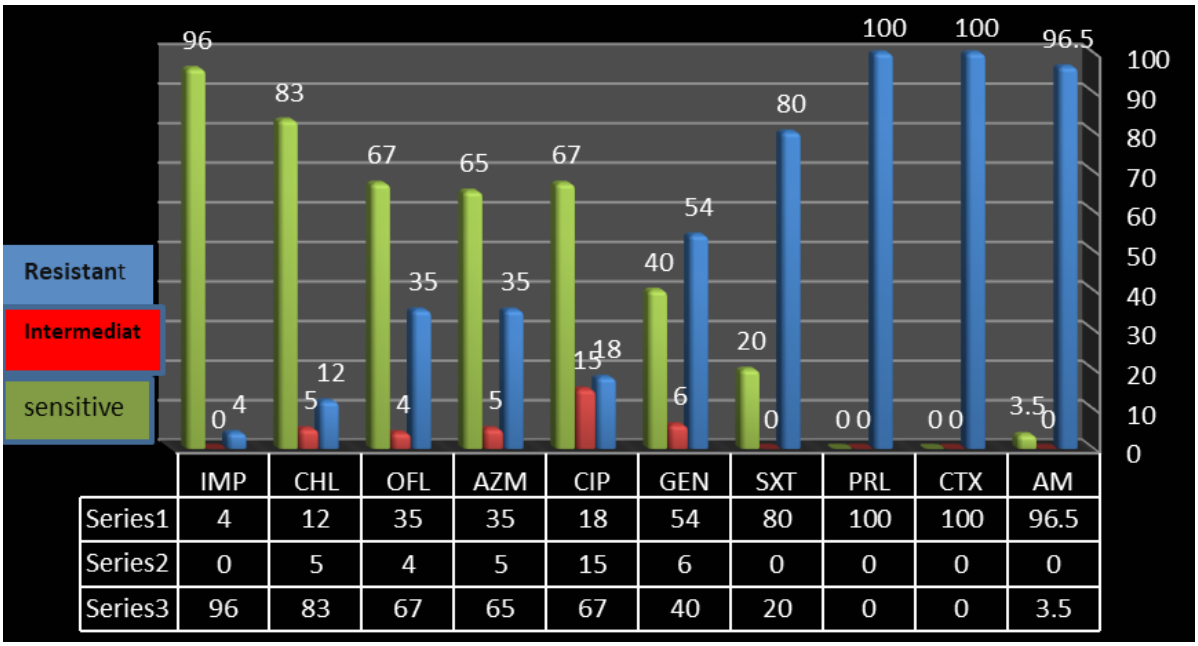
Intereastingly, our results are consistent with the findings of Mehr *et al.* (2017) study where it showed resistance to Chloramphenicol by 14%. The results of the current study also in agrrement with the results of the Algarawyi (2016) study that found the resistance to Piperacillin was 91.1%; however it is inconsistent with the results of Vasaikar *et al*. (2017) study that reported the resistance to Piperacillin was 79%. The results of this study also were consistent with the findings of Chasemian *et al*. (2018) study that isolated *K. pneumonia* which isolated from several hospitals in Tahran and found that the resitance to Cefotaxime was 94.6%; although our results not consistent with the results of Babakhani *et al.,* 2015 study where the resiatance to Ofloxacin was 86%.

Moreover, the current results were also in agreement with the results of Mustafa (2018) study which showed that the resistance of *K. pneumonia* isolated from different clinical cases and from several hospitals in Baghdad were 32% and 84 against Ofloxacin Cefotaxim respectively. It is also consistent with Zedan-ALobadi study whose studied the resistance of *K. pneumonia*, isolated from several hospitals in Baghdad, against various antibiotics; it was revealed that the resistance rate was 2.5% for Imipenem, 95% for Piperacillin, 97% for Ampicillin, and 77.5 for Ttimethoprim.

The genetic relationship was determined by genotyping of the studied bacteria using the Enterobacterial Repetitive Intergenic Consensus (ERIC) method. The results of the current study showed the presence of a genetic relationship between *K pneumoniaea* isolates and also the presence of 12 genotype; the molecular weight of these bundles ranged between (100-1000) base pair as shown in Table (1). Furthermore, the results as shown in Figure (3) found the presence of two clones, each of which contains similar genotypes and found between them a genetic affinity, while the rest of the isolates contain different genotypes. The first clone contained three isolates (3, 5, 8) isolated from hospitalized patients in Al-Yarmouk Teaching Hospital in Baghdad, and from the patients with urinary tract infection. Additionally, these isolates also showed high resistance to antibiotics including Ampicilin, Cefotaxime, and Piperacilli. While the second clone contained four isolates (8, 9, 5 and 6) and these isolates were with a genetic affinity.

Moreover, these isolated showed high resistance to antibiotics and showed sensitivity to Imipenem. Thus, the genotyping method is important in the field of finding

the genetic relationship, determining its resistance and sensitivity to antibiotics, and determining the most pathogenic strain. The results of this study is in agreement with the findings of Mehr *et al*. (2017) which found that there are 12 genotype of *K. pneumoniae* isolates using ERIC-PCR method. While it is inconsistent with the findings of Wasfi *et al*. (2016) study which detected 21 genotype of *K. pneumoniae* using ERIC-PCR method.



	IMP	CHL	OFL	AZM	CIP	GEN	SXT	PRL	CTX	AM
Series1	4	12	35	35	18	54	80	100	100	96.5
Series2	0	5	4	5	15	6	0	0	0	0
Series3	96	83	67	65	67	40	20	0	0	3.5

Figure (1): The resistance of *Klibsiella pneumoniaea* to various antibiotics

Ampicilin (AM), Cefotaxime (CTX), Piperacillin (PRL), Sulfamethoxazole +Trimethoprime (SXT), Gentamycin (GEN), Ciprofloxacin (CIP), Azithromycin (AZM), Ofloxacin(OFX),Chloramphenicol (CHL), Imipenem(IMP).

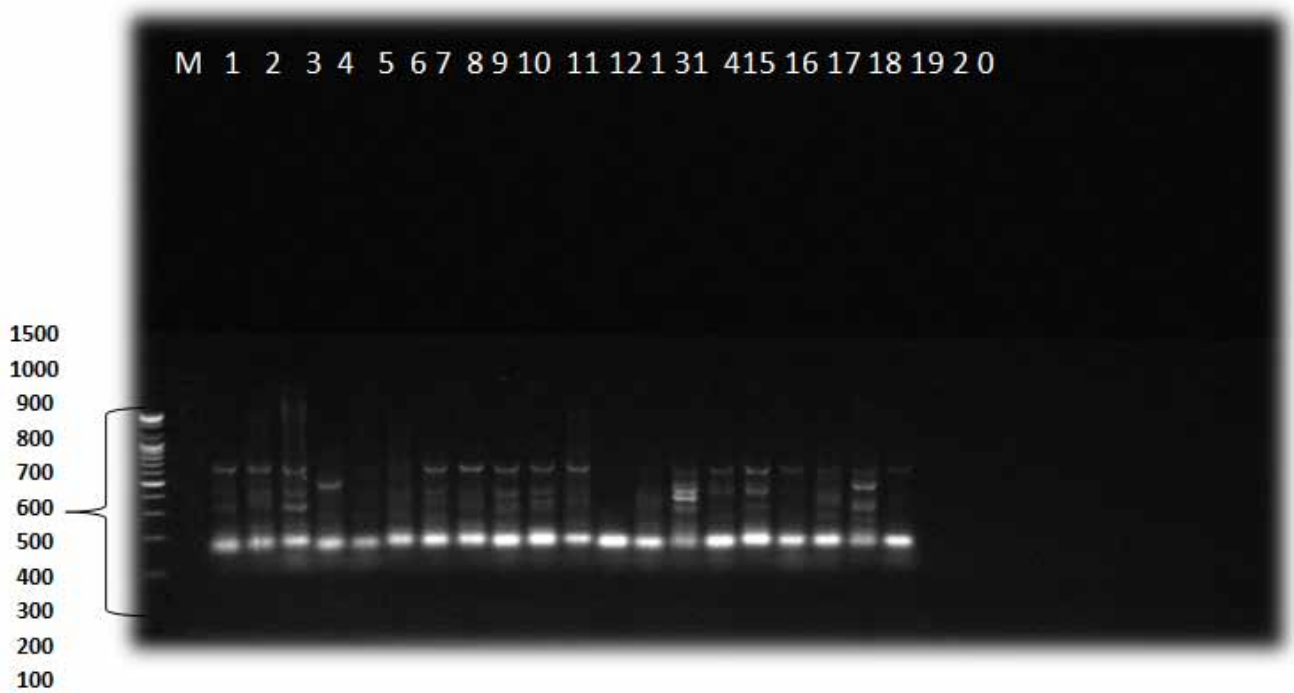


Figure (2): Electrophoresis of PCR product of isolates of *k. pneumoniae* using a specific primers for ERIC genotyping (100-1500 base pairs) on agarose gel (2%) and with voltage difference of 100V for 80 minutes.

Table (1): The Molecular weights and percentages of bundles as products for ERIC-PCR method

Bundles	The molecular weight (bp)	No. of isolates	Percentage (%)
ERIC1	100	1	5
ERIC2	200	8	40
ERIC3	250	2	10
ERIC4	300	7	35
ERIC5	350	8	40
ERIC6	400	5	25
ERIC7	500	3	15
ERIC8	600	13	65
ERIC9	700	2	10
ERIC10	800	1	5
ERIC11	900	1	5
ERIC12	1000	2	10

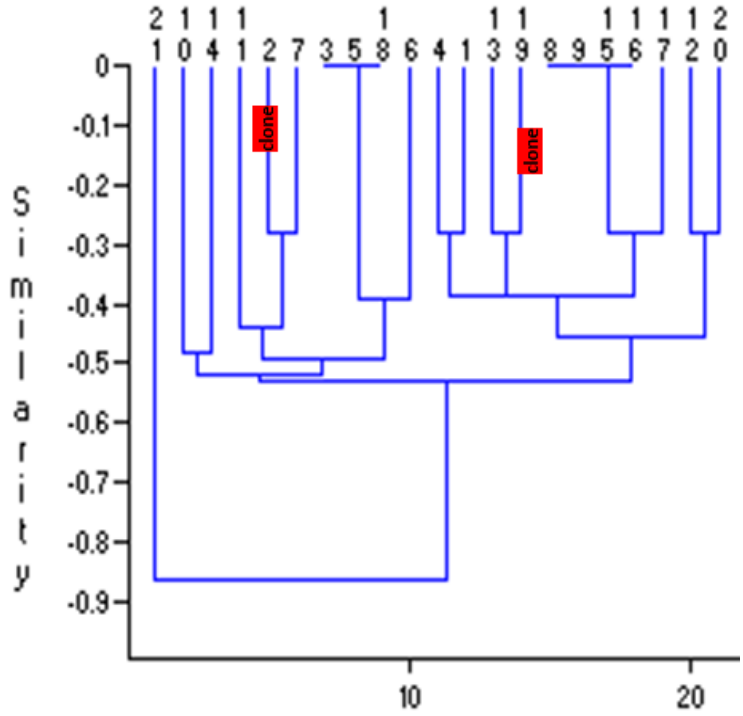


Figure (3): Dendogram of *K. pneumonia* isolates using Past Jaccard/up GMA program.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Ministry of Education and all experiments were carried out in accordance with approved guidelines.

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