

# Detection of Sophistication 2 Integron among *K. pneumoniae* Isolated from Urinary Tract Infections in Al-Najaf Hospitals

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## Abstract

**Background:** Class 2 Integron were most frequently associated with types of Enterobacteriaceae family example *K. pneumoniae*. **Objectives:** The aims were to work out antibiotic susceptibility, resistance patterns and sophistication 2 Integron gene among *K. pneumoniae* Isolated from UTI Infections in Al-Najaf hospitals/Iraq. **Materials and methods:** one hundred and fifty three *K. pneumoniae* isolates (non-duplicate) were "collected from patients admitted to" the middle Kidney diseases and trasplantation in Al-Najaf during January 2020 to June 2020 and diagnosed to be had tract infections by *K. pneumoniae*, also of these isolated cultured on MacConkey agar, traditional biochemical test were used for primary identification and VITEK-2 system was wont to confirmed *K. pneumoniae* isolates suspected then disc diffusion method wont to identification Multidrug-Resistant and therefore the presence of sophistication 2 Integron was investigaed by PCR. **Results:** The results of the present research elucidate that more than two-thirds of *K. pneumoniae* infections in female patients in comparision with male patients, also in this research, we show that Imipenem displayed most effective antimicrobial agents with sensitivity percent of 94.12%, while the lowest one is Cephalothin with sensitivity percent of 0.00%, and 43 isolates of all *K. pneumoniae* isolates are multidrug-resistant (MDR), of these only 5 isolates are harboring integron-class2 gene. **Conclusions:** High prevalence of MDR *K. pneumoniae* in Al-Najaf hospitals and only 5 of these isolates are harboring integron-class2 gene. Imipenem best antimicrobial agents for *K. pneumoniae*.

**Keywords:** Multidrug-Resistant *K. pneumoniae*, urinary Tract infection

## 1. Introduction

*K. pneumoniae* (*K. pneumoniae*) may be a Gram-negative (G-ve), rod-shaped bacteria [1] it's important in hospitals and nosocomial infections. Furthermore, its Infections are difficult to treat because the existence of different virulence factors example the emergence of antibiotic resistance [2].

Enterobacteriaceae member- *K. pneumoniae* may be a clinically significant pathogen and tend to accumulate multidrug resistance (MDR) genes, It Developed different mechanisms of resistance against many of the antibiotics, thus the limiting of therapeutic choices for treating related infections including tract infections (UTIs) [3]. it's one among the foremost pathogens reported to be involved in UTI infections in 2017 by the planet "Health Organization's (WHO) Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of latest Antibiotics [4] Resistant bacterial strains" are one among the foremost important causes of a rise within the rate of antibiotic resistance [5]. Bacteria isolated from hospital-acquired infections have emergency resistance to many antibiotics.

Epidemiological studies have shown that bacteria (*K. pneumoniae*) isolated from hospitals infections have a high resistance to many antibiotics [6].

The widespread antibiotics and misuse of them could also

be liable for the event of resistant strains [7]. Multidrug-resistant strains carry a good range of antimicrobial-resistant genes (i.e. difficult treating strains thanks to harboring resistant genes) [8].

Extended-"spectrum beta-lactamases (ESBLs) are enzymes particularity hydrolyze all beta-lactams including third-generation cephalosporins" except carbapenems and cephamycins [9] Development of antimicrobial resistance, as cefotaximases (CTX-M) enzymes production and *K. pneumoniae* carbapenemases (KPC), Furthermore, the rapid spread of *K. pneumoniae* ESBLs producers may pose a serious global health threat [10].

Mobile genetic elements (MGE) that carrying the beta-lactam resistant genes which were widely spread resistant among Enterobacteriaceae strains cause a rise of ESBLs resistant strains of *K. pneumoniae* [11] Most ESBLs originate in *K. pneumoniae* belong to class A which the sulfhydryl reagent variable (SHV) sort of beta-lactamases [12].

Integrations are "considered powerful mobile genetic elements that are located on plasmids, transposons and pathogenicity islands which facilitate their transferring among different bacteria.

Integrations have a good dispersion among clinically isolated bacteria, according to current publications, and their mobility has become a severe problem in antibiotic resistance in clinical specimens [13] Class 1 integrations are

the most common and have been found in gram negative bacteria, including *K. pneumoniae*, in clinical isolates. Two conserved sections, "the 3' conserved segment (3' CS) and the 5' conserved segment (5' CS), as well as internal gene cassettes that encode antimicrobial resistance genes, make up the structure of class 1 integrons" [14] In *K. pneumoniae*, class-2 integrons are occasionally have found, and sophistication-3 integrons are uncommon [10]. As a result, the goal of this study was to find out how common integron class-2 is in MDR *K. pneumoniae* isolates.

## 2. Materials and Methods

### Patients and Bacterial isolates

one hundred and fifty-three of (non-duplicate) *K. pneumoniae* isolates from clinical specimens at Al-Sader Medical City, Najaf, Iraq, during January 2020 to June 2020. All patients admitted to the middle of kidney diseases and transplantation in Al-Najaf and diagnosed to be had UTI by *K. pneumoniae* during the study.

one hundred and five isolates were from female and Fourty eight isolates from male, these were recovered from midstream urine and indwelling catheter. Urine specimens were obtaining from patients with tract infections (UTIs) ("indwelling catheter, sterile syringe was inserted in to catheter at a 45° angle, then 20–30 mL of urine was withdrawn") and picked up in sterile containers. MacConkey agar (Himedia, India) medium was used for isolation. The growing bacterial colonies were identified such as *K. pneumoniae*, after culturing and incubation at 37 °C for twenty-four hrs. Traditional biochemical test were used for primary identification. All *K. pneumoniae* suspected colonies were confirmed, by using VITEK-2 system (Biome'rieux, France).

### Antibiotic susceptibility testing

*K. pneumoniae* isolates were "tested for susceptiblity to antimicrobial agents and identification of MDR strains were done by using disk diffusion method described by the Clinical and Laboratory Standards Institute (CLSI) guidelines [15]. *K. pneumoniae* isolate which showed resistance to quite three antibiotic classes were identified as MDR" [16].

**Table 2.1 Antibiotics Discs Used in This Study**

Antibiotic	Concentration µg/disc	Manufacturer Company
Ciprofloxacin	5	Oxoid, UK
Ampicillin, Nalidixic acid Cephalothin, Cefotaxime, Aztreonam, Ceftazidime, Ceftriaxone, Cefepime, Cefotaxime	30	
Gentamicin Imipenem	10	
Amoxicillin/Clavulanic acid	20/10	

### DNA extraction

Template DNA that used for polymerase chain reaction, were prepared by using "Wizard genomic DNA purification kit (Promega, USA)". The extracted DNA from each *K. pneumoniae* isolate was stored at -20°C.

### Primer type and preparation

Integron Class 2 primer (intI2), was prepared consistent with the instruction of the manufacturers' instructions and therefore the guideline in Dillon et al. [17] (Table 2.2).

**Table (2.2) Oligonucleotide sequence of Integron Class 2 based on Dillon, et al.,(17)**

Target	Primer Name	Oligonucleotide sequence	Product size bp
intI-2 gene	intI-2 F	5'-CACGGATATGCGACAAAAAGGT-3'	789
	int-I-2 R	3'-GTAGCAAACGAGTGACGAAATG-5'	

Thermocycler Biosystem was used for PCR amplification, table 2.3 and 2.4. The reaction

mixture final volume was 25 microlitter.

**Table 2.3 Monoplex PCR Protocol (Using Promega Go-Tag Green Master Mix**

PCR reaction Mixture	Reaction Mixture Volume/µl
Master mix 2X	12.5
Forward primer	2
Reverse primer	2
DNA template	5
PCR grade water	upto 25

**Table 2.4 Thermocycling condition for amplification of Integron Class 2 based on Dillon, et al.,(17)**

Gene name	Temperature (C°)/ Time				
	Initial denaturation	"Cycling condition"			Final Extention
		"Denaturation	Annealing	Extension"	
intI-2	94/5	94/30sec	62/30sec	72/1min	72/10min

		s	
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### 3. Results and Discussion

- The results of this study show that K. pneumoniae isolates from UTI in female patients (No. 105, 68.6%) were quite male patients (No. 48, 31.4%). The results illustrate that quite two-thirds of infections in female patients as compared with male patients (Figure 3-1).

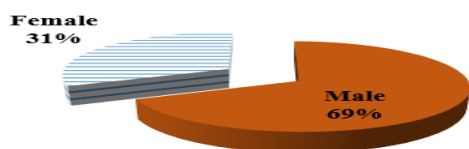


Figure 3.1 Distributions of Urinary Tract Infections among Patients Consistent with The Sex.

These results could also be thanks to the physiological differences between males and females. Several studies confirmed this result, Behzadi et al. [18] published those female patients quite males (i.e. different results were recorded, "January-March 2006:K. pneumoniae caused 13.3%of UTIs(38% in men and 62%in women,January-March 2007:K.pneumonia"caused 9.3%of UTIs(57.1% in

men and 42.9% in women, April-June 2006: K. pneumoniae caused 6.8% of UTIs(41.7% in men and 58.3% in women, April-June 2007:K. pneumoniae caused 11.1% of UTIs(37.9% in men and 62.1% in women, July-September 2006:K. pneumoniae caused 12.4%of UTIs(34% in men and 76.1%in women,July-September 2007:K. pneumoniae caused 11.5%of UTIs(63.2% in men and 36.8%in women,October-December-2006:K. pneumoniae caused 13.2%of UTIs(48.8% in men and 51.2% in women, October-December 2007:K. pneumoniae caused 14.5% of UTIs(15.7% in men and 84.3% in women). Alzahrani et al. [19] reported that females occupied the very best proportions(60%) as compared with males(40%).

The leads to Table 3.1 illustrate that the foremost effective antimicrobial agent is Imipenem (IPM) with a sensitivity percent of 94.12%, while rock bottom one is Cephalothin with a sensitivity ratio of 0.00%. Other antimicrobial agents occupied different ratios of sensitivity, there are 28.1%, 39.87%, 29.41%,67.97%, 57.52%, 60.13%, 79.74%, 37.91%, and 69.93% for Ampicillin, Aztreonam, Amoxicillin-Clavulanic acid, Cefotaxime, Ceftazidime, Gentamicin, Cefoxitin, NegGram, and Ciprofloxacin, respectively.

Table 3.1 Antibiotic Susceptibility expressed by 153 K. pneumoniae isolates

Antibiotic	Resistant		Intermediate susceptibility		Sensitive	
	No.	%	No.	%	No.	%
Ampicillin (AMP)	102	66.67	8	5.23	43	28.10
Amoxicillin-clavulanic acid (AMC)	107	69.93	1	0.65	45	29.41
Aztreonam (ATM)	92	60.13	0	0.00	61	39.87
Cephalothin (CEF)	153	100.00	0	0.00	0	0.00
Cefotaxime (CTX)	46	30.07	3	1.96	104	67.97
Ceftazidime (CAZ)	61	39.87	4	2.61	88	57.52
Cefoxitin (FOX)	31	20.26	0	0.00	122	79.74
Imipenem (IPM)	8	5.23	1	0.65	144	94.12
Gentamicin (GEN)	61	39.87	0	0.00	92	60.13
Nalidixic acid (NAL)	93	60.78	2	1.31	58	37.91
Ciprofloxacin (CIP)	46	30.07	0	0.00	107	69.93

Only an honest sensitivity to meropenem (98.60 percent), Piperacillin-tazobactam was seen among K. pneumoniae isolates when it came to biofilm formation(90.00percent), and amikacin(95.80 percent),K. pneumoniae on the other hand, showed good sensitivity to levofloxacin,meropenem,ciprofloxacin,piperacillin-tazobactam and amikacin with sensitivity of 100.00 percent, 95.83 percent, 91.67 percent, 87.50 percent, and 86.67 percent, respectively, among non-biofilm producing isolates [20].

K. pneumoniae isolates were reported to be (100%)immune of ampicillin and (100%) sensitive to amikacin by Madahiah et al. [21].The resistance rates for amoxicillin-clavulanic acid and ciprofloxacin were(36.69and 38.75)%respectively. According to Cepas et al. [22] Forty of K. pneumoniae were resistant to

amoxicillin-clavulanic acid and ciprofloxacin The most important element in antimicrobial resistance is antibiotic exposure. Antibiotic resistance is being fueled by a variety of reasons,contain the use of antibiotics in hospitals and within communities , agriculture, animal and even in environment production. As a result of the ability to buy "antibiotics" for free without a prescription, antibiotics are overused. In the health services setting, it is very likely that extensive and extended use of antibiotics is the basic idea about the widespread prevalence of hospital infections that are resistant to antibiotics that are difficult to treat [23].

Results in Table 3.2 demonstrate that 43 isolates of all K. pneumoniae isolates are multidrug-resistant (MDR). of those only 5 isolates are harboring integron-class2 gene tables (3-3) and Fig(3-2).

Table 3.2 Resistance patterns of 43 multidrug-resistant K. pneumoniae strains collected from UTI patients in Al-Najaf province.

Pattern of Resistance	No. of isolates
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AMP, AMC, ATM, NAL, CEF	12
AMP, AMC, ATM, CIP, CEF, CAZ	4
AMP, ATM, IMP, CAZ, CEF	3
AMP, ATM, NAL, GEN, CEF, CAZ	9
AMC, ATM, FOX, NAL, CEF, CIP	5
AMC, ATM, FOX, CEF, CTX	8
Resistant to all antibiotics in this study	2
Total no. of MDR isolates	43

**Table 3.3 Association of Resistance Patterns and Genes among Integron-Class 2 Positive Isolates of K. pneumoniae**

MDR K. pneumoniae isolate	Antibiotic Resistance Patterns
K. p 22	AMP, AMC, NAL, CEF
K. p 86	AMP, ATM, CIP, CEF
K. p 97	ATM, IMP, CAZ, CEF
K. p 132	AMP, ATM, NAL, GEN, CEF, CAZ
K. p 145	FOX, NAL, CEF, CIP
K.p = K. pneumoniae	

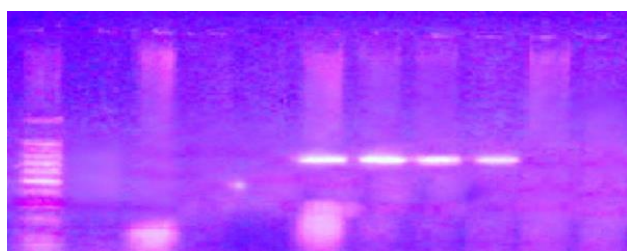


Figure 3.2: Ethidium-Bromide Stained Monoplex Amplification Products of Integron-Class 2 Gene, Electrophoresed at 70V/cm, in 1.5 Agarose.

#### Lane M: DNA ladder Marker

According to Nirwati et al. [20] the total proportion of MDR K. pneumoniae isolates is (54.49) percent. Cepas et al., [22] also reported a significant percentage of MDR K. pneumoniae strains, stating that 38% of K. pneumoniae were MDR. Another investigation, GM et al. [24] found that 37 isolates (90.2%) were MDR. The majority of MDR K. pneumoniae isolates were also found to be resistant to fluoroquinolone, penicillin, cephalosporin, sulfonamide and aminoglycoside. This result is consistent with the results of a recent study by Moini et al. [25], who found that (46.6%) of K. pneumoniae isolates were MDR, indicating strong resistance to 3rd "generation cephalosporins, ampicillin and aminoglycosides. This great challenge in managing infections".

## 4. Conclusions

The results of our research revealed that:

- 1-high prevalence of MDR K. pneumoniae in Al-Najaf hospitals.
- 2-Only Five bacteria of all MDR K. pneumoniae harboring integron-class 2 gene.
- 3- UTI commonest infection that happens in women in comparison with men.
- 4- Imipenem best anti-microbial agents for K. pneumoniae.

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#### Ethical Approval

The research obtained the ethical approval issued by the medical laboratories department in college of health and Medical Technologies/Kufa, Middle Euphrates University and verbal consent was obtained from all participants.

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