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Prevalence of SUL(1,2), GYR(A, B) and OXA genes among multidrug resistance *Klebsiella pneumoniae* isolates recovered from women suffering urinary tract infection

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ABSTRACT

Klebsiella pneumoniae is a significant concern multidrug-resistant microorganism and a one common gram negative bacteria associated with infections of women urinary tract. Therefore, this work aimed to the molecular screening of Sul(1and 2), Gyr(A and B) and OXA genes among *K. pneumoniae* isolates in Najaf City, Iraq. Out of 250 urine specimens were collected from women showing symptoms of urinary tract infection during five months January to of May 2019, bacterial growth was157 isolates, included 133 gram negative compared with 24 gram positive bacteria while 98 specimens were no growth. According to the Vitek-2 system, 30 *K. pneumoniae* isolates were obtained. Data on current work revealed that the 26-35 age group was the highest 14 *K. pneumoniae* isolates. Results of antimicrobial susceptible recorded all isolates were multi-drug resistant (MDR) and they have a different range of resistance. However, all 30 isolates(100%) resistant to ampicillin drugs, while the lowest rate was 1(3.33%) for Imipenem drug. PCR assay revealed exist of oxa, sul-1, sul-2, gyr-A and gyr-B genes among *K. pneumoniae* isolates with rates 20(66.66%), 11(36.66%), 22(73.33%), 3(10%) and 17(56.66%) respectively.

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INTRODUCTION

Urinary tract infection (UTIs) has become of the more prevalent bacterial infections, which impact nearly one hundred and fifty million person every year globally. *Klebsiella pneumoniae* isolate has been a major opportunistic microorganism that involved in infections of the human urinary tract. It has many virulence factors such as capsule, adherence factors, lipopolysaccharide, iron acquisition, as well as biofilm formation (Flores-Mireles *et al.*, 2015).

Previously, there was an enormous index of antibacterial agents such as beta-lactam drugs, sulfonamides, fluoroquinolones as well as aminoglycosides were more successful in creating the desired result or limiting and controlling the infections occur by human gram negative pathogen, but last years, this pathogen involved *K. pneumoniae* showed emerging concern in multiple drug resistance in worldwide. At same respect, drug resistance mostly results from overuse and misuse of antimicrobial agents badly decrease the effectiveness of these drugs, leading to growing complication in *K. pneumoniae* therapy (Franci *et al.*, 2015; Fernandes and Martens, 2017; Caneiras *et al.*, 2019).

However, bacterial resistance to drugs usually due to different mechanisms, one important which

exploited by the pathogen is catalyzed by enzymes encoded in genes harbored in chromosome or plasmid -mediated. Therefore, this work aimed to investigate the spreading of some genes which have related to a resistance of bacteria to some drugs.

MATERIALS AND METHODS

Bacterial specimens

The current study was focused on the role and rate of *K. pneumoniae* among bacterial urinary tract infection for women that attended to clinical laboratories in Najaf city, Iraq, during five months from the beginning of January to ending of May 2019, were collected 250 urine specimens from non-duplicate women patients.

Bacterial cultivation and identification

A total of 250 urine specimens were obtained randomly from women suspected to have urinary tract infection with old ranged from 16 to 50 years, where all urine specimens were collected by sterile container, the immediately transported to bacteriology laboratory in Faculty of Science, University of Kufa, then cultured on sterile blood agar and MacConkey agar plates and overnight incubated at 37Cº under aerobic condition.

All bacterial growth were identified according to microscopic morphology, lactose ferment, motility, oxidase test as well asIMViC tests and all suspected isolates were finally identified using Vitek-2 system (Macfaddin and Odds, 2000; Shlash and Tuwaij, 2018).

Antibacterial agents assay

Current work involved testing susceptibility of different commercial classes of antimicrobial agents (bioanalysis, Tuekey) against 30 isolates of *K. pneumoniae* on the surface of muellerhinton agar according to methods of Kirby-bauer (Bauer *et al.*, 1966). The resistance, intermediate and sensitive of isolates were limited depending on the instructions of the Clinical and Laboratory Standards Institute (CLSI, 2017). All antimicrobial disks with their concentration enrolled in Table 5.

Total DNA extraction

Total genomic DNA of were extracted from overnight liquid growth for 30 isolates of *K. pneumoniae* using a genomic extraction kit (Favorgen, Biotech Corp., Korea), where the extraction was performed depending on the protocol of the company. All DNA was stored under -20C° condition using deep freezing until done PCR to the detection of oxa, Sul-1, Sul-2, gyr-A and gyr-B genes using specific primers and conditions listed in Table 2. The apparatus of gel document system (Cleaver, United Kindom), was used to see and migrate of PCR product after stained the gel with ethidium bromide.

Statistical analysis

The Significance statistical and Comparing among data of the current study was analyzed according to Fisher's exact test (Graph pad prism version 10).

RESULTS AND DISCUSSION

Patients specimens

Results of bacterial growth on culture media among 250 non-duplicate patients have UTIs, were revealed that the rate of bacterial growth in gram negative was 133 (53.2%) while gram positive bacteria reached to 24 (9.6%), at the same time, 93 (37.2%) was no bacterial growth (Table 3).

K. pneumoniae identification and age groups

According to results of morphology, biochemical test as well as vitek-2 system, among 133 (53.2%) gram negative bacteria obtained only 30 (22.55%) isolates identified as *K. pneumoniae* (Table 4).

Data of Table 4 was appeared that *K. pneumoniae* able to cause infection to various woman age groups but at different rates. The highest rate of infection was 14 (27.45%) between 26-35 ages, while the lowest rate observed between 46-50 ages reached to 1 (7.69%).

Susceptibility of *K. pneumoniae* isolates using the disk diffusion method

This work also involved study the susceptibility of 30 *K. pneumoniae* isolates against different classes of antibacterial agents. At the same respect, the data revealed that all 30 *K. pneumoniae* isolates were resistance to three different antibiotic classes and considered as multi-drug resistance (MDR). However, the resistance of this pathogen was high resistances included 30(100%) Ampicillin,27(90%) Cefotaxime, 26(86%), Ceftazidame, while Tetracycline and Trimethoprim reached to 24(80%). Lowest rate of resistance found in Impenem, Meropenem and Netilmicin reached to 1(3.33%), 5(16.66%) and 10(33.33%) respectively. Others antibacterial agents give resistance ranged from 13(43.33%) to 20(66.66%) as shown in Table 5.

Molecular investigation

According to data analysis of PCR products, this study revealed that oxa gene has high frequency among *K. pneumoniae* isolates, out of 30 isolates found 20(66.66%) harbor positive band for the bla-oxa gene (Figure 1). At same respect, the results

Table 1: Specific primer sequence used in this work

Gene	Name	Sequence (5´to 3´)	Product size(bp)	Reference
oxa	oxa-F	ATATCTCACTGTTGCATCTCC	618	Karami *et al.* (2008)
	oxa-R	AAACCCTTCAAACCATCC		
sul-1	sul1-F	GGATGGGATTTTTCTTGAGCCCCGC	308	Wain *et al.* (2003)
	sul1-R	ATCTAACCCTCGGTCTCTGGCGTCG		
sul-2	sul2-F	TCAACATAACCTCGGACAGT	707	Chu *et al.* (2001)
	sul2-R	GATGAAGTCAGCTCCACCT		
gyr-A	gyrA-F	ATGGCTGAATTACCTCAATC	398	Sierra *et al.* (2002)
	gyrA-R	GTGTGATTTTAGTCATACGC		
gyr-B	gyrB-F	CAAACTGGCGGACTGTCAGG	345	Ling *et al.* (2003)
	gyrB-R	TTCCGGCATCTGACGATAGA		

Table 2: PCR conditions used in this work

PCR gene	Temperature (c) / Time					
	Initial	Cycling condition			Final	Cycle Number
	denaturation	Denaturation	Annealing	Extension	extension	
oxa	94 C°/5min	94 C°/1min	55 C°/1min	72 C°/ 1min	72 C°/ 10min	30
sul-1	94 C°/5min	94 C°/1min	57.5 C°/1min	72 C°/ 1min	72 C°/ 2min	30
sul-2	94 C°/5min	94 Cº/1min	50 C°/1min	72 C°/ 1min	72 C°/ 7min	30
gyr-A	94 C°/5min	94 C°/45sec	50 C°/45 sec	72 C°/ 1min	72 C°/5min	30
gyr-B	94 C°/5min	94 C°/1min	62 C°/1min	72 C°/ 2min	72 C°/ 1min	30

Table 3: Characteristics of Bacterial culture from urine specimens

Bacterial culture	Number (percentage)	P value
Gram positive	24 (9.6%)	< 0.0001
Gram negative	133 (53.2%)***	
No growth	93 (37.2%)	
Total	250 (100%)	

Table 4: Distribution of *K. pneumoniae* isolates according to woman age groups

Age groups (year)	Gram negative bacteria	*K. pneumoniae* isolate	P value
16-25	28(21.05%)	6 (21.42%)	0.0002
26-35	51(38.34%)	14 (27.45%)***	
36-45	41(30.82)	9 (21.95%)	
46-50	13(9.77%)	1 (7.69%)	
total	133(100%)	30 (22.55%)	

Table 5: Antimicrobial susceptibility of *K. pneumoniae* isolates

Antibiotic disk	Sensitive	Intermediate	Resistance
Ampicillin (AMP, 10 μg)	0(0%)	0(0%)	30(100%)
Piperacillin (PRL, 100μg)	7(23.33%)	3(10%)	20(66.66%)
Cefoxitn (FOX, 30 μg)	11(36.66%)	4(13.33%)	15(50%)
Cefotaxime (CTX, 30 μg)	3(10%)	0(0%)	27(90%)
Ceftazidame (CAZ, 30 μg)	3(10%)	1(3.33%)	26(86%)
Cefepeme (FEP, 30 μg)	9(30%)	1(3.33%)	20(66.66%)
Aztreonam (ATM, 30 μg)	8(26.66%)	2(6.66%)	20(66.66%)
Netilmicin (NET, 10 μg)	18(60%)	2(6.66%)	10(33.33%)
Amikacin (AK, 10 μg)	12(40%)	1(3.33%)	17(56.66%)
Tobromycin (TM, 10μg)	9(30%)	3(10%)	18(60%)
Ciproflaxacin (CIP,10 μg)	11(36.66%)	5(16.66%)	14(46.66%)
Impenem (IMP, 10 μg)	28(93%)	1(3.33%)	1(3.33%)
Meropenem (MEM, 10 μg)	24(80%)	1(3.33%)	5(16.66%)
Tetracycline (TE, 30 μg)	6(20%)	0(0%)	24(80%)
Doxycycline (DO, 30 μg)	15(50%)	2(6.66%)	13(43.33%)
Trimethoprim (TMP, 5μg)	4(13.33%)	2(6.66%)	24(80%)

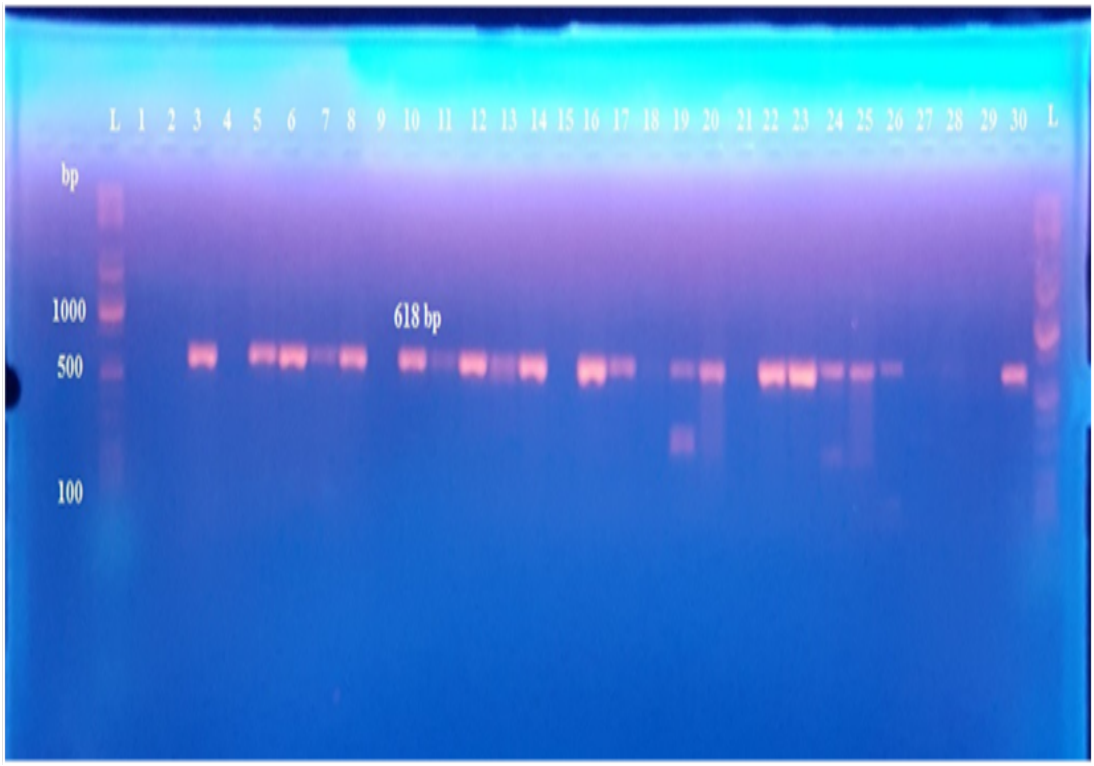

Figure 1: Amplification product of *bla-oxa* gene among 30 isolates of *K. pneumoniae*

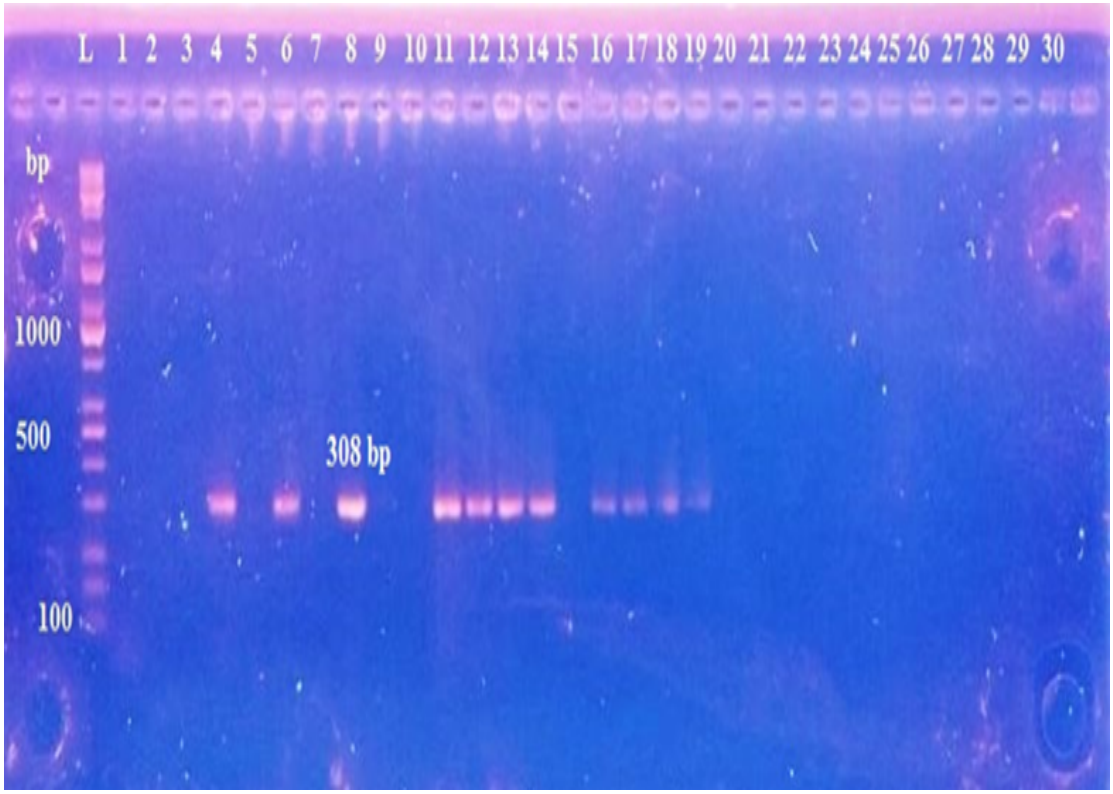

Figure 2: Amplification product of Sul-1gene among 30 isolates of *K. pneumoniae*

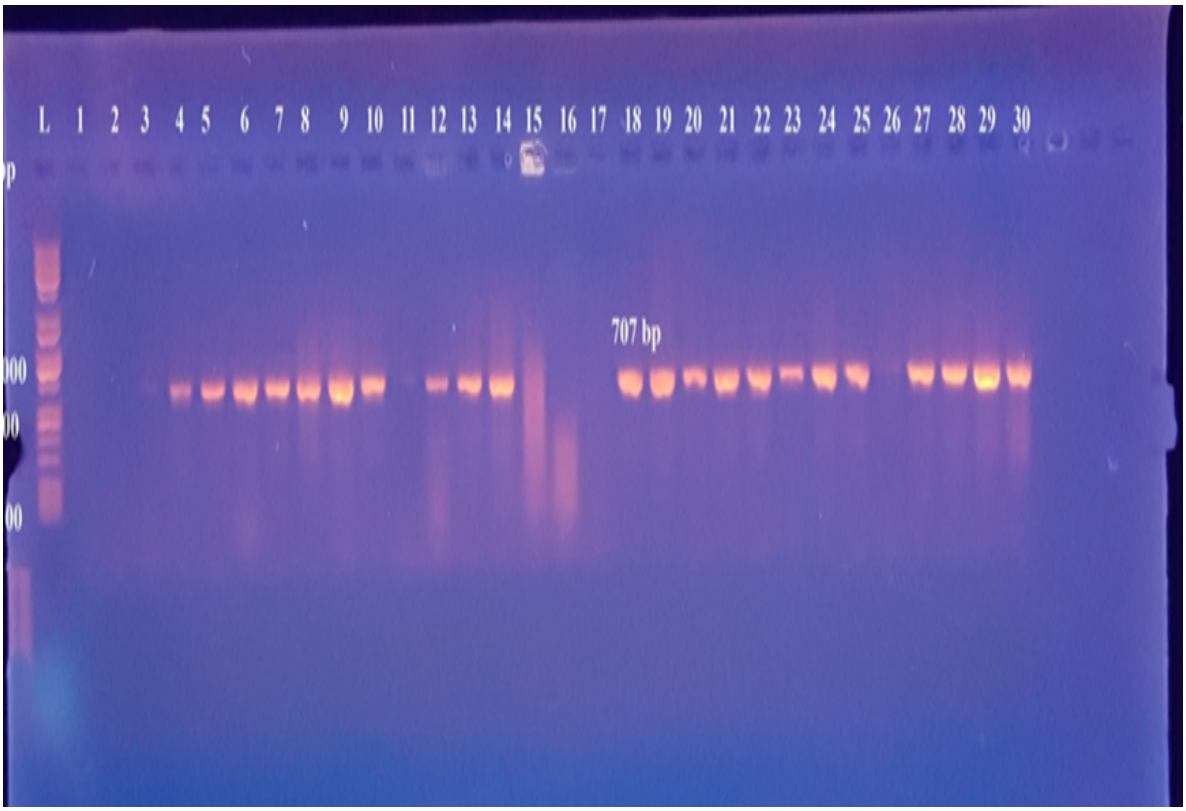

Figure 3: Amplification product of Sul-2 gene among 30 isolates of *K. pneumoniae*

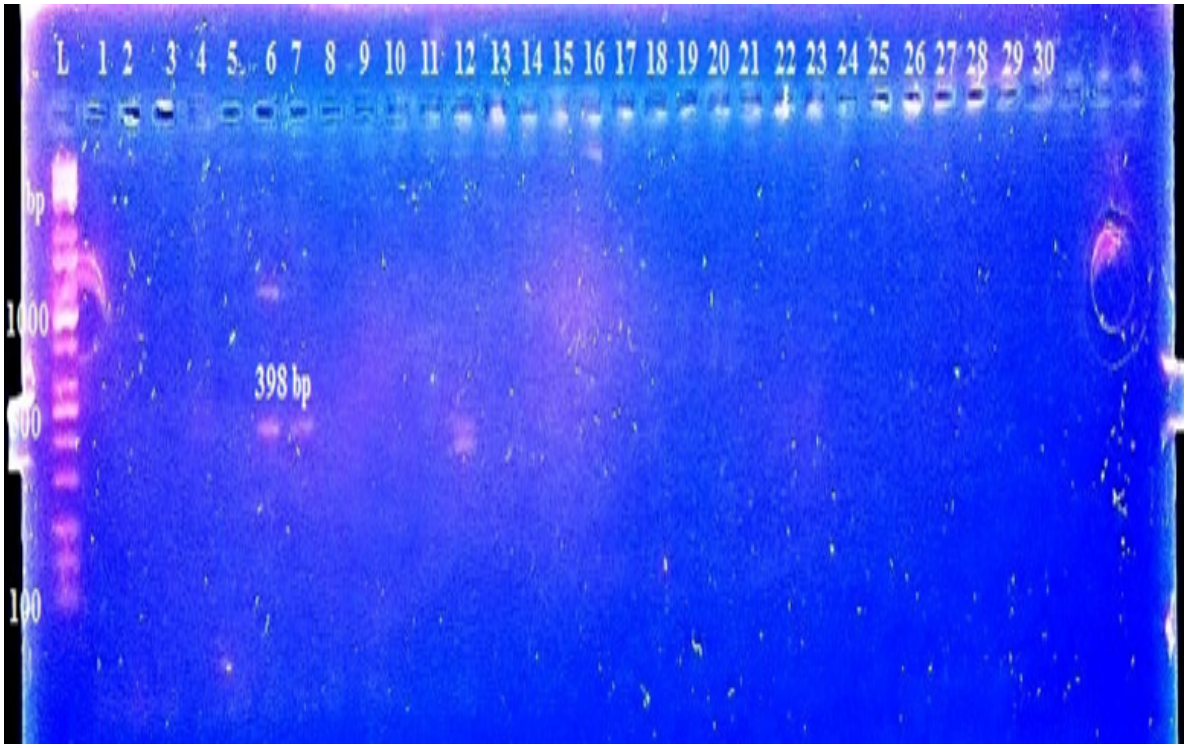

Figure 4: Amplification product of Gyr-A gene among 30 isolates of *K. pneumoniae*

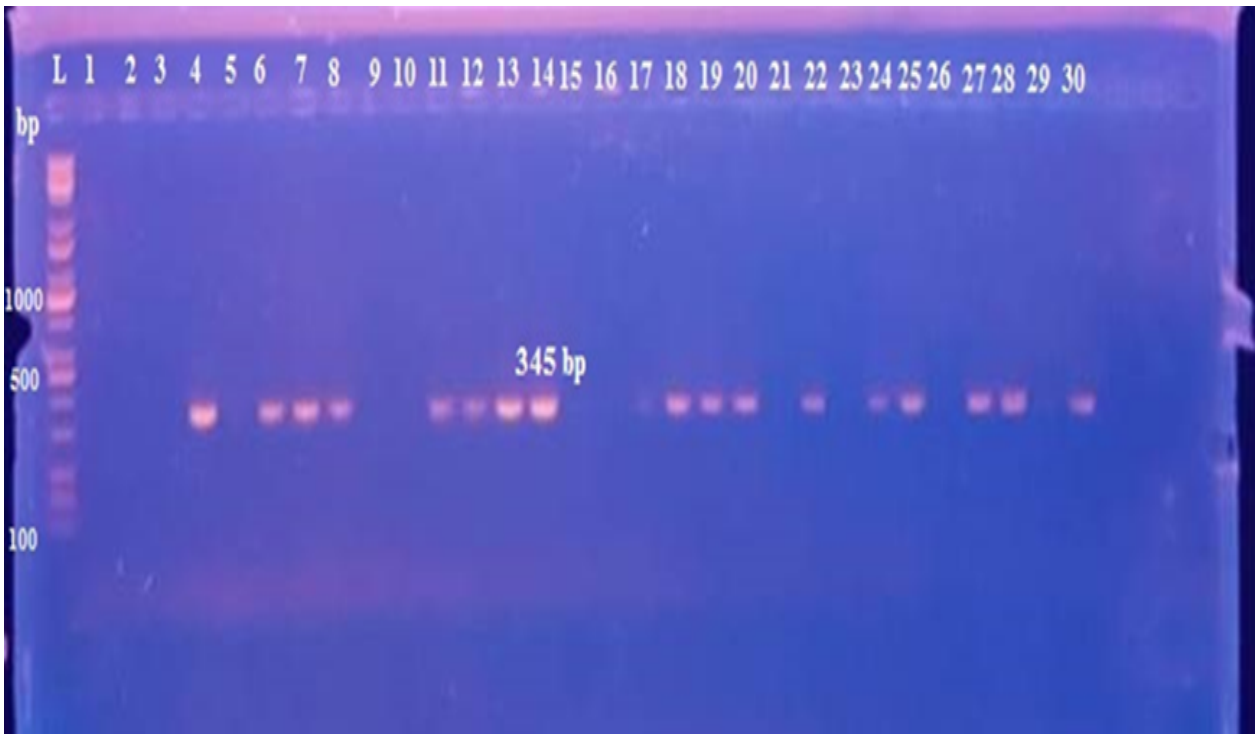

Figure 5: Amplification product of Gyr-B gene among 30 isolates of *K. pneumoniae*

Table 6: Phenotypic and genotypic characteristics of *K. pneumoniae* isolates

No	Phenotypic characteristics (Antimicrobial agent resistance)	Genotypic characteristics
1	AMP, FOX, TMP,AK,TE	-
2	AMP, CAZ, CTX, TMP, AK,NET,CIP,TE	-
3	AMP, PRL, FOX, CAZ, CTX, FEP,TE,DO	OXA,
4	AMP, PRL, FOX, CAZ, CTX, FEP,AZM, TMP,TM, AK,CIP, TE,DO	Sul-1,Sul-2, GyrB
5	AMP, CAZ, CTX, FEP, TMP, AK, CIP,TE,DO	OXA, Sul-2
6	AMP, CAZ, CTX, FEP, TMP,TM, AK, TE,DO	OXA,Sul-1,Sul-2,GyrA,GyrB
7	AMP, CAZ, CTX, FEP,AZM, TMP,TM, CIP, TE,DO	OXA,Sul-2,GyrA,GyrB
8	AMP, CTX, FEP,AZM, TMP,TM,	OXA, Sul-1 ,Sul-2,GyrB
9	AMP, CAZ, , CTX, AZM, TM, TE	Sul-2
10	AMP, PRL, FOX, CAZ, CTX, FEP,AZM, TMP,TM, AK,NET, CIP, TE,DO	OXA,Sul-2
11	AMP, PRL, FOX, CAZ, CTX, FEP,AZM, TMP,TM, AK, CIP,TE	OXA, Sul-1, GyrB
12	AMP, CAZ, CTX, FEP, TMP,TM, AK,NET,MEM,IMP, CIP,TE	OXA,Sul-1,Sul-2,GyrA,GyrB
13	AMP, PRL, FOX, CAZ, CTX, TMP,TE,DO	OXA, Sul-1 ,Sul-2,GyrB
14	AMP, PRL, FOX, CAZ, CTX, FEP,AZM, TMP,TM,MEM, CIP,TE	OXA, Sul-1 ,Sul-2,GyrB
15	AMP, PRL, CAZ, CTX, FEP,AZM, TM, AK,NET,TE	-
16	AMP, PRL, FOX, CTX, FEP,AZM, TMP,	OXA, Sul-1
17	AMP,FOX,CAZ,CTX,FEP,AZM,TMP,TE,DO,	OXA, Sul-1
18	AMP,PRL,FOX,CAZ,CTX,FEP,AZM,TMP,TM,AK,NET,MEM,TE,	Sul-1,Sul-2, GyrB
19	AMP,PRL,CAZ,CTX,FEP,AZM,TMP,AK,TE,	OXA,Sul-1,Sul-2,GyrB
20	AMP,PRL,CAZ,CTX,FEP,AZM,TMP,TM,AK,NET,CIP,TE,DO	OXA,,Sul-2,GyrB
21	AMP,FOX, TMP,TE,DO	Sul-2
22	AMP,PRL,CAZ, CTX,AZM, TMP,TM, AK, CIP, TE,DO	OXA, Sul-2,GyrB
23	AMP,PRL,FOX, CAZ, CTX, FEP,AZM, TMP,TE	OXA, Sul-2
24	AMP,PRL,FOX, CAZ, CTX, FEP,AZM, TMP,TM, AK,NET, CIP,TE	OXA, Sul-2,gyrB
25	AMP,CAZ,CTX, TM,MEM	OXA, Sul-2,GyrB
26	AMP,PRL, FOX, CAZ, CTX,AZM,	OXA,
27	AMP,CAZ,CTX, TMP,TM, AK,NET, CIP,	Sul-2,GyrB
28	AMP,CAZ,CTX, FEP,AZM, TMP,TM, AK,NET, CIP, TE,DO	Sul-2, GyrB
29	AMP,PRL,FOX, AZM,MEM	Sul-2
30	AMP,PRL,FOX, CAZ,CTX, TMP,TM, AK,NET, CIP, TE,DO	OXA,Sul-2, GyrB

proved that *K. pneumoniae* isolates have both sul-I and sul-2 genes but at different rates reached to 11(36.66%) and 22(73.33%) respectively(Figures 2 and 3). On other hands, this work was investigated the frequency of gyr-A and gyr-B genes using specific primers among 30 multi-drug resistance *K. pneumoniae* isolates which reached to 3(10%) and 17(56.66%) for gyr-A and gyr-B genes respectively (Figures 4 and 5).

At same respect, 6 revealed that 2 isolates of *K. pneumoniae* (no. 6 and 12) have five genes and three isolates were negative for all tested genes in this study.

Bacterial isolates were involved in this work only predominant growth while mixed non- predominant growth was excluded. The data showed among 250 urine specimens, 30 isolates belong to *K. pneumoniae* and this result corresponds with several local and global previous studies (Aljanaby *et al.*, 2018). In Najaf city, a study done by Shlash and Tuwaij(2018) they indicated that out of 200 urine specimens obtained 40 isolates of *K. pneumoniae*. The current study revealed that gram negative bacteria isolated more than gram positive bacteria this may be due to they are endogenous as well as exogenous. However, this data agree with (Foxman, 2014) who mention The causative of UTIs infections originate from Gram-negative bacteria, particularly Enterobacteriaceae, and some Gram-positive pathogen.

One of the main aim of current work was estimation of antibiogram profile for 30 isolates of uropathogenic *K. pneumoniae*. The phenotypic data as mention in Table 5 revealed that this pathogen has high antimicrobial resistance. However, without doubt this not unique research that obtained high rate s of drug resistances but there are several local studies indicated to spreading of multi-drug resistance among gram negative , at same respect there is a group of etiologies and factors that interfere with each other as well as natural or acquired resistance to bacteria, taking the drug without the advice of a competent doctor and non-adherence to the appropriate dose encouraged resistance between microbes

Universal and locally, there were many papers reported that oxa genes a significant found among gram negative bacteria (Al-Muhanna *et al.*, 2016; Abrar *et al.*, 2019; Ranjbar *et al.*, 2019). High frequency of this gene made bacterial resistance to ranges of penicillins, some cephalosporin generations and sometime extend to involve carbapenem drugs (Antunes and Fisher, 2014).

Sulfa drugs are a group of synthesized medicine agents which still wide used in treatment of urinary tract infections through effect on bacterial growth (bacteriostatic drugs) by prevent synthesis of folic acid in bacteria (Tacic *et al.*, 2017). Here, this work focused on distribution of sulfonamide-resistant genes included sul-1 and sul-2 genes among 30 MDR *K. pneumoniae* isolated from women suffering from UTI. However, both genes sul-1 and sul-2 were 11(36.66%) and 22(73.33%) respectively. This rate was more than a previous local study achieved by (Hayder and Aljanaby, 2019) they found among 30 MDR *Citrobacter freundii* isolated from UTI patients, rate of sul-1 and sul-2 genes were 7(23%) and11(36.6%) respectively. Also this rate was higher than a study done by (Shin *et al.*, 2015) who found, rate of sul-1 and sul-2 genes were 2(18.8%) and 5(31.2%) among 15 *K. pneumoniae* isolates.

At the same respects, the results of molecular assays about DNA gyrase genes were estimated. gyr-B gene was high frequency compared with gyr-A gene. One reason may be due to continuous pressure to overuse of antimicrobial agents involved macrolide groups in Iraq led pathogen to acquired or mutation to resistance this medicine. However, A study achieved by (Hou *et al.*, 2015). In china, they mention among 38 multidrug-resistance *K. pneumoniae* isolated from different sources found 27 isolates harbored gyrA gene while DNA gyrase gene (gyr-B), not detection. However, the data of PCR as shown in Table 6 appeared that 3 isolates of *K. pneumoniae* no. 1, 2 and 15 were MDR but negative for all tested genes this maybe return to other mechanisms for drugs resistance.

CONCLUSIONS

Klebsiella pneumoniae is a significant contagious among women that suffering from urinary tract infection, which has a high resistance to most antibacterial agents except imipenem drug remains the most efficient antibacterial agent against this pathogen. Molecular assay appeared that *K. pneumoniae* harbored oxa, sul-1, sul-2, gyr-A and gyr-B genes with different rates.

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