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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2017.217.232

Research Article
Phenotypic and Molecular Characterization of Multidrug Resistant *Klebsiella pneumoniae* Isolated from Different Clinical Sources in Al-Najaf Province-Iraq

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Abstract

Background and Objective: Burns infections and urinary tract infections are the most important prevalent diseases in Asian countries, such as Iraq. *Klebsiella pneumoniae* is one of the most important bacteria cause this type of infections especially in hospitals. Therefore, the aim of this study was to investigate the prevalence of multi-drug resistance *K. pneumoniae* and extended-spectrum beta-lactamases producing *K. pneumoniae* isolates from inpatients with urinary tract infection and burns infections in Al-Kufa hospital in Al-Najaf province, Iraq. **Materials and Methods:** A total of 285 clinical samples were collected from in-patients infected with urinary tract infection (141 urine samples) and burns infections (144 burns swabs). Fourteen different antibiotics were used by disc diffusion method and 13 antimicrobials resistance genes were used by PCR technique. **Results:** A total of 43 *K. pneumoniae* strains were isolated. The highest resistance rate was observed for amoxicillin 25 μg and amoxicillin+clavulanic acid 20+10 μg (97.67%) while the lowest resistance rate was observed for imipenem 10 μg (9.30%). The most common resistance associated-genes were *blaSHV* (86.04%) and at lower prevalence were IMP (9.30%). **Conclusion:** *Klebsiella pneumoniae* strains isolated from burns infections were more virulent than those isolated from urinary tract infections.

Key words: *Klebsiella pneumoniae*, multidrug-resistant, ESBL, resistance associated-genes, UTI, burns infections, Iraq

Received: March 04, 2017 Accepted: April 03, 2017 Published: April 15, 2017

Citation: Ahmed Abduljabbar Jaloob Aljanaby and Haneen Mohammed Reda Jaber Alhasnawi, 2017. Phenotypic and molecular characterization of multidrug resistant *Klebsiella pneumoniae* isolated from different clinical sources in Al-Najaf province-Iraq. Pak. J. Biol. Sci., 20: 217-232.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Klebsiella pneumoniae is one of the most important multidrug-resistant (MDR) opportunistic Gram-negative bacteria cause different illness with high mortality and morbidity due to hospital-acquired infections and non-hospital-acquired infections such as, pneumonia, Urinary Tract Infection (UTI), burns infections and bacteremia[1]. Recently, *K. pneumoniae* became more resistant to antimicrobials especially to third generating cephalosporins and many studies focused on isolation of this pathogen from patients infected with different infections[2-4].

Klebsiella pneumoniae is one of the most Enterobacteriaceae strains can produce Extended-Spectrum Beta-lactamases Enzymes (ESBLs) and become highly effective against different beta-lactam antimicrobials. On the other hand, Extended-Spectrum Beta-lactamase (ESBL) producing bacteria are resistant to various antimicrobials classes lead to difficult to treat diseases and called MDR bacteria[5].

Multidrug-resistant bacteria and ESBL producing *K. pneumoniae* and other Gram-negative bacteria have worldwide distributions with high degree of prevalence in both hospitals and community[6-8]. Nearly about more than 390 different types of ESBLs have been recognized around the world, among which *blaSHV*, *blaTEM* and *blaCTX-M* were more prevalent[9]. Moreover, MDR and ESBL producing *K. pneumoniae* strains isolated from both inpatients and outpatients can cause treatment failure with different antimicrobials therapy such as, beta-lactam, cephalosporins, aminoglycosides and others[10,11]. The rapid emergence of ESBL producing some gram negative bacteria like *K. pneumoniae* has significantly increased ($p<0.05$) in the risk of developing serious nosocomial infections worldwide[12,13].

In Iraq (as a developing country) it is difficult to assess the accurate incidence of some infections like, urinary tract infection and burns infections besides bacterial resistance due to underreporting, lack of surveillance as well limited published data. Therefore, this study was designed to investigate the prevalence of *K. pneumoniae* strains in urinary tract infection and burns infections as a causative agent among patients at Kufa Central Hospital, Kufa, Iraq and explore their antimicrobial resistance patterns, which may constitute an epidemiological importance regarding the wide-spread of MDR and ESBL producing bacteria in this country.

MATERIALS AND METHODS

Samples collection: Two hundred and eighty five clinical samples were collected, 141 urine samples were collected from in-patients infected with UTI and 144 burn swabs were collected from patients infected with burns infections admitted to the AL-Kufa hospital in AL-Najaf province-IRAQ during the period from July, 2016 to January, 2017.

Culture: All urine samples (midstream urine and after cleaned the genitals) were collected in sterile disposable containers (Himedia-India) and centrifuged (Memmert-Germany) 2000 rpm for 2 min. Immediately, the sediment incubated with brain hart infusion broth (Oxoid-UK) at 37°C overnight and streaked by sterile swab (Bioanalyse-Turkey) on blood agar (Oxoid-UK) surface and MacConkey agar (Oxoid-UK) surface and incubated aerobically overnight at 37°C[14]. Burns swabs were collected from site of infection and incubated with brain hart infusion broth (Oxoid-UK) at 37°C overnight for activation and then streaked immediately by sterile swab (Bioanalyse-Turkey) on blood agar surface (Oxoid-UK) and MacConkey agar (Oxoid-UK) surface and incubated aerobically overnight at 37°C[14].

Identification: All developing colonies on the surface of agar plates were identified by standard bacteriological methods according to MacFaddin[15]. In addition to, all suspected *K. pneumoniae* isolates were streaked by sterile swab (Bioanalyse-Turkey) on Chrome agar surface (Orientation Company-France). Vitek2® system (BioMerieux® -France) was used for final identification.

Antimicrobial susceptibility testing: Fourteen different antimicrobials were used in this study according to Kirby-Bauer method and the Clinical Laboratory Standards Institute (CLSI)[16] was used as a guideline as follow: one colony from each *K. pneumoniae* strain was grown in Mueller Hinton broth (Oxoid,UK) at 37°C for 24 h. *Klebsiella pneumoniae* cultures were adjusted according to McFarland nephelometer scale (1.5×10^8 CFU mL^{-1}) and streaked on Mueller Hinton agar (Oxoid, UK) surface by sterile swab (Bioanalyse-Turkey). Antimicrobial susceptibility and resistance was determined by strain growth zone diameter according to CLSI guidelines[16]. The following 14 antimicrobials were used, AX: Amoxicillin 25 μg, AMC: Amoxicillin+Clavulanic acid 20+10 μg, CTX: Cefotaxime 30 μg, CRO: Ceftriaxone 30 μg, CAZ: Ceftazidime 30 μg, IMP: Imipenem 10 μg, CN: Gentamicin 15 μg, AK: Amikacin 30 μg, TM: Tobramycin 10 μg, TE: Tetracycline 30 UI, DO: Doxycycline 30 μg, CIP: Ciprofloxacin 5 μg, C: Chloramphenicol 30 μg and F: Nitrofurantoin 30 μg. All these antimicrobials were provided from (Bioanalyse-Turkey). *Escherichia coli* ATCC 25922 strain was used as controls. Any bacterial strain resist to a minimum at least 3 different classes of antibiotics it is MDR, any bacterial strain remain susceptible

to only one or two class of antibiotics it is Extensive-Drug Resistance (XDR) and any bacterial strain resistance to all sub classes in all classes of antibiotics it is Pan-Drug Resistance (PDR) (CLSI)[16].

Primary test for production of extended spectrum β-lactamase: This method was done according to CLSI[17] as follows: Antibiotic susceptibility testing was done to three types of antimicrobials (3rd generation cephalosporins): CAZ (30 μg), CTX (30 μg) and CRO (30 μg) . If inhibition zone for bacterial isolates were: ≤27 mm for CAZ, ≤22 mm for CTX and ≤25 mm for CRO, this result considered as positive result for production of extended spectrum beta lactamase.

Confirmatory test for extended spectrum β-lactamase: This test was performed according to Sarojamma and Ramakrishna[18] as follows. Augmentin disc (AMC 30 μg) was placed in the center of Mueller Hinton agar plate (Oxoid, UK). Around of three sides of AMC (30 μg) disc, a disc of CAZ (30 μg), CTX (30 μg) and CRO (30 μg) were placed with distance of fifteen mm from center to center of AMC (30 μg) disc. Then the plate was incubated overnight at 37°C. The result was considered as positive results for production of ESBL if inhibition zone was increased towards the AMC (30 μg) disc.

DNA extraction: Method of Yang *et al.*[19] was used to extraction of total DNA as follows: Five pure and fresh colonies of *K. pneumoniae* strains were suspended in 200 μL of sterile deionized water and cells were placed in water bath (Memmert-Germany) at 100°C for 30 min, immediately the solution was placed in ice for 30 min and the other cellular components was removed by centrifugation at 9000 rpm for 15 min. Finally the supernatant was used as the DNA template.

Polymerase Chain Reaction (PCR) detection of antimicrobials resistance-associated genes: Polymerase chain reaction was used to detect 14 antimicrobials resistance-associated genes include: β-lactamase genes (*blaTEM, blaSHV, blaCTX-M, blaCTX-M-1* group, *blaCTX-M-2* group, *blaCTX-M-8* group, *blaCTX-M-9* group and *blaCTX-M-25* group), quinolone resistance-associated genes (*qnrA*, *qnrB* and *aac(6')-Ib-cr*), aminoglycoside resistance-associated genes (*aacC1* and *aacC2*) and penems resistance-associated gene (*blaIMP*). All primers used in this study and all PCR thermo cycling conditions are listed in Table 1 and 2, respectively. All PCR products were loaded on a 1.5% (w/v) agarose gel with 0.5 mg mL^{-1} safestain and were analyzed by gel electrophoresis.

Table 1: Sequencing of primers used in PCR for 14 antimicrobials resistance-associated genes of *K. pneumoniae*

Genes	Oligo sequences (3'→5')	Product size (bp)	References
β-lactamase genes			
blaTEM	F: CAGCGGTAAGATCCTTGAGA		
	R: ACTCCCCGTCGTGTAGATAA	643	Ensor *et al.*[20]
blaSHV	F: GGCCGCGTAGGCATGATAGA		
	R: CCCGGCGATTTGCTGATTTC	714	
blaCTX-M	AACCGTCACGCTGTTGTTAG		
	TTGAGGCGTGGTGAAGTAAG	766	
blaCTX-M-1	GCGTGATACCACTTCACCTC		
	TGAAGTAAGTGACCAGAATC	260	Xu *et al.*[21]
blaCTX-M-2	TGATACCACCACGCCGCTC		
	TATTGCATCAGAAACCGTGGG	341	
blaCTX-M-8	F: TTTGCCCGTGCGATTGG		
	R: CGACTTTCTGCCTTCTGCTCT	368	Gao *et al.*[22]
blaCTX-M-9	F: ATGGTGACAAAGAGAGTGCA		
	R: CCCTTCGGCGATGATTCTC	870	Messai *et al.*[23]
blaCTX-M-25	F: TTGTTGAGTCAGCGGGTTGA		
	R: GCGCGACCTTCCGGCCAAAT	490	Huang *et al.*[24]
Quinolone resistance-associated genes			
qnrA	ATTTCTCACGCCAGGATTTG GATCGGCAAAGGTTAGGTCA	627	Robicsek *et al.*[25]
qnrB	GATCGTGAAAGCCAGAAAGG ACGATGCCTGGTAGTTGTCC	469	
aac(6')-Ib-cr	TGACCAACAGCAACGATTCC		
	TTAGGCATCACTGCGTGTTC	554	Shams *et al.*[26]
Aminoglycoside resistance-associated genes			
aacC1	F: ATGGGCATCATTCGCACATGTAGG		
	R: TTAGGTGGCGGTACTTGGGTC	873	Hujer *et al.*[27]
aacC2	F: ATGCATACGCGGAAGGCAATAAC		
	R: CTAACCGGAAGGCTCGCAAG	861	
Penems resistance-associated gene			
blaIMP	F:GGAATAGAGTGGCTTAATTCTC		
	R:CCAAACCACTACGTTATC	624	Kaczmarek *et al.*[28]

Table 2: Thermo cycling conditions of PCR for 14 antimicrobials resistance-associated genes of *K. pneumoniae*

	Initial denaturation		Cycling condition					
Genes	Temperature (°C)	Time (min)	Denaturation	Annealing	Extension	No. of cycles	Final extension	References
TEM	95°C	5 min	94°C/30 sec	52°C/45 sec	72°C/45 sec	30	72°C/7 min	Ensor *et al.*[20]
SHV	95°C	5 min	94°C/30 sec	55°C/60 sec	72°C/45 sec	30	72°C/7 min	
CTX-M	95°C	5 min	94°C/30 sec	57°C/45 sec	72°C/45 sec	30	72°C/7 min	
CTX-M-1	94°C	4 min	94°C/1 min	54°C/60 sec	72°C/1 min	30	72°C/10 min	XU *et al.*[21]
CTX-M-2	95°C	5 min	95°C/50 sec	56°C/40 sec	72°C/60 sec	35	72°C/5 min	
CTX-M-8	95°C	5 min	95°C/50 sec	50°C/40 sec	72°C/60 sec	35	72°C/5 min	Gao W *et al.*[22]
CTX-M-9	95°C	5 min	95°C/50 sec	50°C/40 sec	72°C/60 sec	35	72°C/5 min	Messai Y *et al.*[23]
CTX-M-25	94°C	5 min	94°C/25 sec	53°C/40 sec	72°C/60 sec	30	72°C/6 min	Huang *et al.*[24]
qnrA	95°C	5 min	94°C/1 min	55°C/1 min	72°C/2 min	30	72°C/5 min	Robicsek *et al.*[25]
qnrB	95°C	5 min	94°C/1 min	55°C/1 min	72°C/2 min	30	72°C/5 min	
aac(6')-Ib-cr	95°C	5 min	94°C/1 min	55°C/1 min	72°C/2 min	30	72°C/5 min	Shams *et al.*[26]
aacC1	95°C	5 min	94°C/1 min	55°C/1 min	72°C/2 min	30	72°C/5 min	Hujer *et al.*[27]
aacC2	95°C	5 min	94°C/1 min	55°C/1 min	72°C/2 min	30	72°C/5 min	
blaIMP	95°C	5 min	95°C/50 sec	50°C/1 min	72°C/1min	35	72°C/5 min	Kaczmarek *et al.*[28]

Table 3: Totals and percentages of bacterial isolates collected from in-patients with urinary tract infections and burns infections

	Burn swabs		Urine samples		Total	
Isolates	No.	%	No.	%	No.	%
Gram negative	92	32.28	121	42.45	213	74.73
Gram positive	52	18.24	11	3.85	63	22.12
No growth	0	0.0	9	3.15	9	3.15
Total	144	50.53	141	49.47	285	100

Table 4: Total and percentages of *K. pneumoniae* strains isolated from in-patients with burns infections and urinary tract infections

	Total samples		*K. pneumoniae*	
Site of isolates	No.	%	No.	%
Burn infection	92	43.19	19	8.92
Urinary tract infection	121	56.81	24	11.26
Total (100%)	213	100	43	20.18

Statistical analysis: Fisher's exact test was used in this study for the comparison between samples by using SPSS software version 6. The p values less than the 0.05 level of significance were considered statistically significant[28].

RESULTS

Total isolates: According to gram stain, the results proved that out of the 285 total specimens there were 213 specimens (74.73%) were gram negative bacteria, 63 specimens (22.12%) were gram positive bacteria and there were 9 specimens (3.15%) with no any bacterial growth (Table 3).

Also, the result demonstrated that out of the 213 total Gram-negative bacteria, there were 43 strains (20.18%) it has been diagnosed as *K. pneumoniae*, 19 strains (8.92%) isolated from burns infections and 24 strains (11.26%) isolated from UTI (Table 4, Fig. 1).

Antimicrobial susceptibility testing: This test was performed for 43 *K. pneumoniae* strains. The results of the present study demonstrated that the antimicrobial resistance rates of the

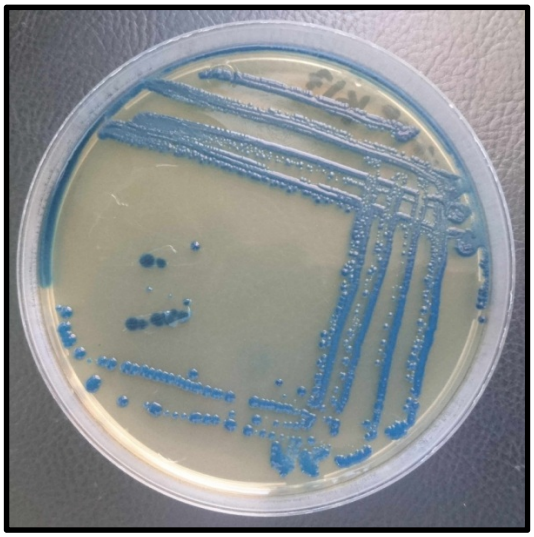

Fig. 1: Metallic blue colonies of *K. pneumoniae* on chrome agar surface after 24 h at 37°C of incubation (Selective test)

43 strains to amoxicillin 25 μg, amoxicillin+clavulanic acid 20+10 μg, cefotaxime 30 μg, ceftriaxone 30 μg, nitrofurantoin 30 μg and ceftazidime 30 μg were all high (97.67-90.69%). The moderate resistance rate was observed for doxycycline 30 μg, tetracycline 30 UI, gentamicin 15 μg and chloramphenicol 30 μg with percentage ranged from 48.83-44.18% . The lowest resistance rate was observed for amikacin 30 μg (25.58%) and

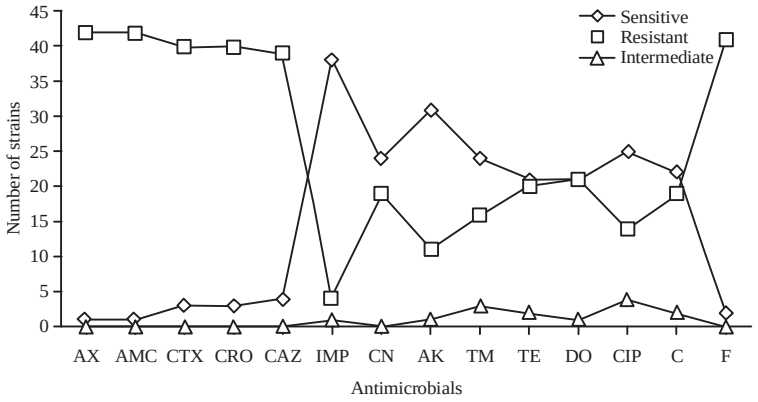

Fig. 2: Antimicrobials sensitivity test of 43 *K. pneumoniae* strains isolated from in-patients with urinary tract infections and burns infections

AX: Amoxicillin 25 μg, AMC: Amoxicillin+Clavulanic acid 20+10 μg, CTX: Cefotaxime 30 μg, CRO: Ceftriaxone 30 μg, CAZ: Ceftazidime 30 μg, IMP: Imipenem 10 μg, CN: Gentamicin 15 μg, AK: Amikacin 30 μg, TM: Tobramycin 10 μg, TE: Tetracycline 30 UI, DO: Doxycycline 30 μg, CIP: Ciprofloxacin 5 μg, C: Chloramphenicol 30 μg, F: Nitrofurantoin 30 μg

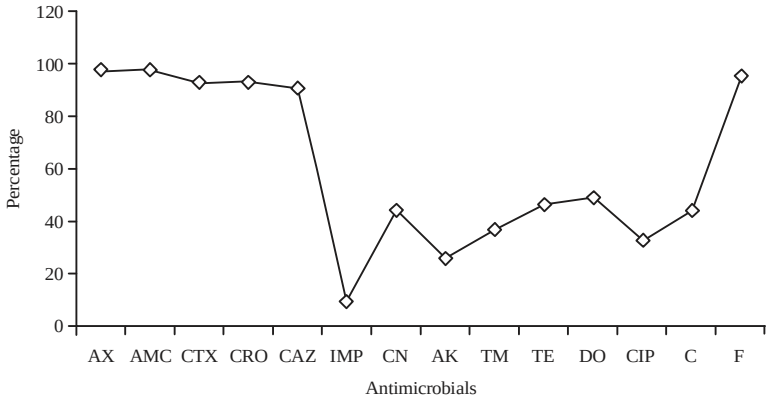

Fig. 3: Total percentage of antimicrobials resistance of 43 *K. pneumoniae* strains isolated from in-patients with urinary tract infections burn infections

AX: Amoxicillin 25 μg, AMC: Amoxicillin+Clavulanic acid 20+10 μg, CTX: Cefotaxime 30 μg, CRO: Ceftriaxone 30 μg, CAZ: Ceftazidime 30 μg, IMP: Imipenem 10 μg, CN: Gentamicin 15 μg, AK: Amikacin 30 μg, TM: Tobramycin 10 μg, TE: Tetracycline 30 UI, DO: Doxycycline 30 μg, CIP: Ciprofloxacin 5 μg, C: Chloramphenicol 30 μg, F: Nitrofurantoin 30 μg

Table 5: Prevalence of drug-resistant *K. pneumoniae* strains isolated from in-patients with urinary tract infections and burns infections

	Multidrug-resistant*		Extensive drug-resistant		Pandrug-resistant	
Source of isolates: No. (%)	No.	%	No.	%	No.	%
Burns: 19 strain (44.18)	8	42.10	9	47.36	2	10.52
Urine: 24 strains (55.82)	24	100.00	0	0.00	0	0.00
Total: 43 strains (100)	32	74.41	9	20.93	2	4.65

*: $p<(0.05)$

imipenem 10 μg (9.30%) (Fig. 2, 3). Also, the results of the present study proved that there were 32 strains (74.41%) of *K. pneumoniae* were MDR, 9 strains (20.93%) were XDR and 2 strains (4.65%) were PDR (Table 5).

Primary and confirmatory phenotypic tests for production of ESBL: Of the 43 *K. pneumoniae* strains tested for the presence of ESBL-producing strains according to primary and confirmatory phenotypic test, the results demonstrated that 35 strains (81.39%) were ESBL-producing *K. pneumoniae* and 8 strains (18.61%) were non-ESBL-producing *K. pneumoniae* ($p<0.05$) (Fig. 4, Table 6). Phenotypic resistance profile of 43 clinical strains are given in Table 7.

Table 6: Total numbers and percentage of extended spectrum beta lactamase-producing *K. pneumoniae* strains isolated from in-patients with urinary tract infections and burn infections

	Extended spectrum beta lactamase-producing* *K. pneumoniae*		Non extended spectrum beta lactamase-producing *K. pneumoniae*		Total	
Source of isolates: No. (%)	No.	%	No.	%	No.	%
Burn : 19 strains	19	100.00	0	0.00	19	100
Urine: 24 strains	16	66.66	8	33.34	24	100
Total: 43 strains	35	81.39	8	18.61	43	100

*p<(0.05)

Table 7: Antimicrobials resistance profile of 43 *K. pneumoniae* strains isolated from in-patients with urinary tract infections and burn infections

		Isolates			
Source of strain	Phenotypic resistance profiles	No.	%	Resistance types	ESBL
Burns	AX, AMC, CTX, CRO, CAZ, CN AK, TM, TE, DO, CIP, C,F	12	85.71	XDR	+
Burns	AX, AMC, CTX, CRO, CAZ, AK, TM, TE, DO, CIP, C, F	12	85.71	XDR	+
Burns	AX, AMC, CTX, CRO, CAZ, CN AK, TM, TE, DO, CIP, C,F	13	92.58	XDR	+
Burns	AX, AMC, CTX, CRO, CAZ, AK, TM, TE, DO, CIP, C, F	12	85.71	XDR	+
Burns	AX, AMC, CTX, CRO, CAZ, AK, TM, TE, DO, CIP, C,F	12	85.71	XDR	+
Burns	AX, AMC, CTX, CRO, CAZ, AK, TM, TE, DO, CIP, C, F	12	85.71	XDR	+
Burns	AX, AMC, CTX, CRO, CAZ, CN, IMP, AK TM TE, DO, CIP, C, F	14	100.00	PDR	+
Burns	AX, AMC, CTX, CRO, CAZ, CN, IMP, AK,TM, TE, DO,CIP,C,F	14	100.00	PDR	+
Burns	AX, AMC, CTX, CRO, CAZ, CN, AK, TM, TE, DO, CIP, C,F	13	92.58	XDR	+
Burns	AX, AMC, CTX, CRO, CAZ, AK, TM, TE, DO, CIP, C, F	12	85.71	XDR	+
Burns	AX, AMC, CTX CRO, CAZ, CN, AK, TE, DO, CIP, C, F	12	85.71	XDR	+
Burns	AX, AMC, CTX, CRO, CAZ, TM, TE, DO, CIP, C, F	11	78.57	MDR	+
Burns	AX, AMC, CTX, CRO, CAZ, TM, DO, C, F	9	64.28	MDR	+
Burns	AX, AMC, CTX, CRO, CAZ, CN, TM, DO, C, F	10	71.42	MDR	+
Burns	AX, AMC, CTX, CRO, CAZ, CN, TM, DO, C, F	10	71.42	MDR	+
Burns	AX, AMC, CTX, CRO, CAZ, DO, F	7	50.0	MDR	+
Burns	AX, AMC, CTX, CAZ, CN, TM, DO, F	8	57.14	MDR	+
Burns	AX, CAZ, TM, TE, DO, F	6	42.85	MDR	+
Burns	AX, AMC, CTX, CRO, CAZ, IMP,DO, TE, F	9	64.28	MDR	+
Urine	AX, AMC, CTX, CRO, CAZ, CN,TE, F	8	57.14	MDR	+
Urine	AX, AMC, CTX, CRO, CAZ, CN, TE, F	8	57.14	MDR	+
Urine	AX, AMC, CTX, CRO, CAZ, CN, TE, DO, C, F	10	71.42	MDR	+
Urine	AX, AMC, CTX, CRO, CAZ, CN, TE, DO, C, F	10	71.42	MDR	+
Urine	AX, AMC, CTX, CRO, CAZ, CN,TE DO, C, F	10	71.42	MDR	+
Urine	AX, AMC, CRO, CAZ, CN, TE, C, F	8	57.14	MDR	+
Urine	AX, AMC, CTX, CRO, CIP, F	6	42.85	MDR	+
Urine	AX, AMC, CTX, CRO, IMP, CN, CIP, F	8	57.14	MDR	+
Urine	AX, AMC, CTX, CRO, CN, F	6	42.85	MDR	-
Urine	AX, AMC, CTX, CRO, CN, F	6	42.85	MDR	-
Urine	AX, AMC CTX, CAZ, CN, F	6	42.85	MDR	-
Urine	AX, AMC, CTX, CAZ, CN, F	6	42.85	MDR	-
Urine	AX, AMC, CTX, CRO CAZ, F	6	42.85	MDR	+
Urine	AX, AMC, CTX, CRO, CAZ, F	6	42.85	MDR	+
Urine	AX, AMC, CTX, CRO, CAZ, F	6	42.85	MDR	+
Urine	AX, AMC, CTX, CRO, CAZ, F	6	42.85	MDR	+
Urine	AX, AMC, CTX, CRO, CAZ, F	6	42.85	MDR	+
Urine	AX, AMC, CRO, CAZ, F	7	50.00	MDR	-
Urine	AX, AMC, CTX CRO, CAZ, F	6	42.85	MDR	+
Urine	AX, AMC, CTX, CRO, CAZ, F	6	42.85	MDR	+
Urine	AX, AMC, CTX, CRO, CAZ, F	6	42.85	MDR	+
Urine	AX, AMC, CTX, CRO, CAZ	7	50.00	MDR	-
Urine	AX, AMC, CTX, CRO, CAZ	7	50.00	MDR	-
Urine	AMC, CTX, CRO, CAZ, F	7	50.00	MDR	-

AX: Amoxicillin 25 μg, AMC: Amoxicillin+Clavulanic acid 20+10 μg, CTX: Cefotaxime 30 μg, CRO: Ceftriaxone 30 μg, CAZ: Ceftazidime 30 μg, IMP: Imipenem 10 μg, CN: Gentamicin 15 μg, AK: Amikacin 30 μg, TM: Tobramycin 10 μg, TE: Tetracycline 30 UI, DO: Doxycycline 30 μg, CIP: Ciprofloxacin 5 μg, C: Chloramphenicol 30 μg, F: Nitrofurantoin 30 μg, MDR: Multidrug resistance, XDR: Extensive drug resistance, PDR: Pandrug resistance, ESBL: Extended spectrum beta lactamase, +: Positive, -: Negative, No. (100%): Total numbers (n) and percentage (%) of *K. pneumoniae* strains that were resistant to antimicrobials

Molecular detection of antimicrobials resistance genes: The results of the current study demonstrated that out of the 43 strains there were 24 strains (55.81%) were positive for *blaTEM* gene (Fig. 5), 37 strains (86.04%) were positive for

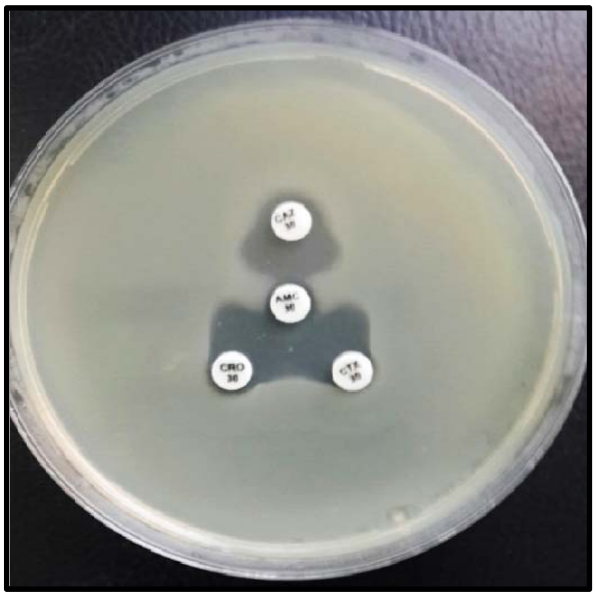

Fig. 4: Positive result of Double Disc Synergy Test for ESBL-producing *K. pneumoniae* on Mueller-Hinton agar surface after 24 h at 37°C of incubation
AMC: Amoxi/Clavulanic acid 30 μg, CTX: Cefotaxime 30 μg, CRO: Ceftriaxone 30 μg, CAZ: Ceftazidime 30 μg

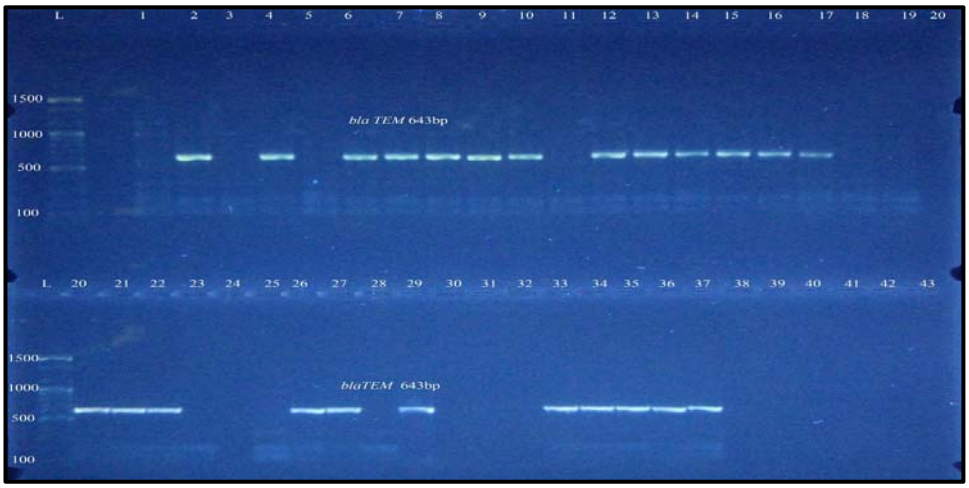

Fig. 5: PCR amplified products from total extracted DNA of 43 *K. pneumoniae* strains. Amplified with *blaTEM* gene show positive results at 643 bp
L: DNA molecular size marker

blaSHV gene (Fig. 6), 22 strains (51.16%) were positive for *blaCTX-M* gene (Fig. 7), 21 strains (48.83%) were positive for *blaCTX-M-1* group gene (Fig. 8), 5 strains (11.62%) were positive for *blaCTX-M-2* group (Fig. 9) and there were no prevalence of *blaCTX-M-8*, *blaCTX-M-9* and *blaCTX-M-25* groups.

Also, the results proved that out of the 43 strains of *K. pneumoniae*, 36 strains (83.72%) were positive for *qnrB* gene (Fig. 10), 33 strains (76.74%) were positive for *aac(6')-Ib-cr* gene (Fig. 11), while there was no prevalence of *qnrA* gene.

On the other hand, the results indicated that there were 12 strains (27.90%) were positive for *aacC1* gene (Fig. 12), 4 strains (9.30%) were positive for *aacC2* gene (Fig. 13) and there were only 4 strains (9.30%) were positive for *IMP* gene (Fig. 14). The prevalence and distribution of antimicrobials resistance genes are given in Table 8 and 9.

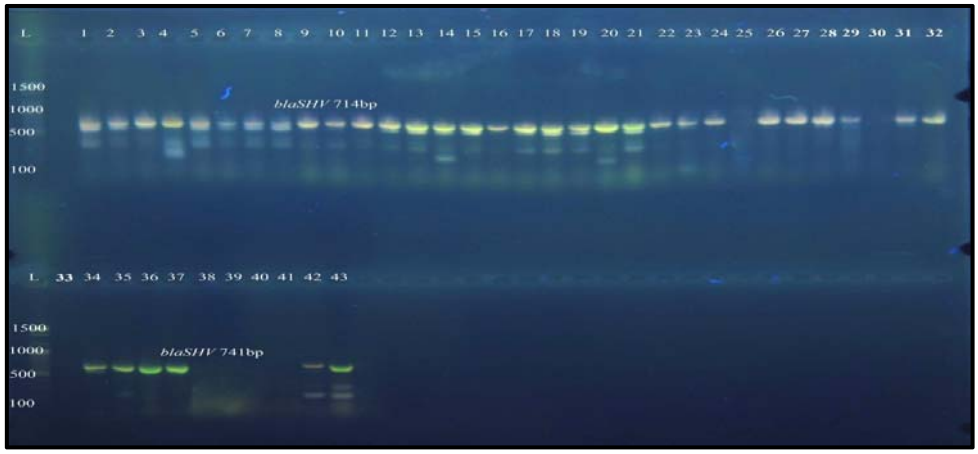

Fig. 6: PCR amplified products from total extracted DNA of 43 *K. pneumoniae* strains. Amplified with *blaSHV* gene show positive results at 714 bp
L: DNA molecular size marker

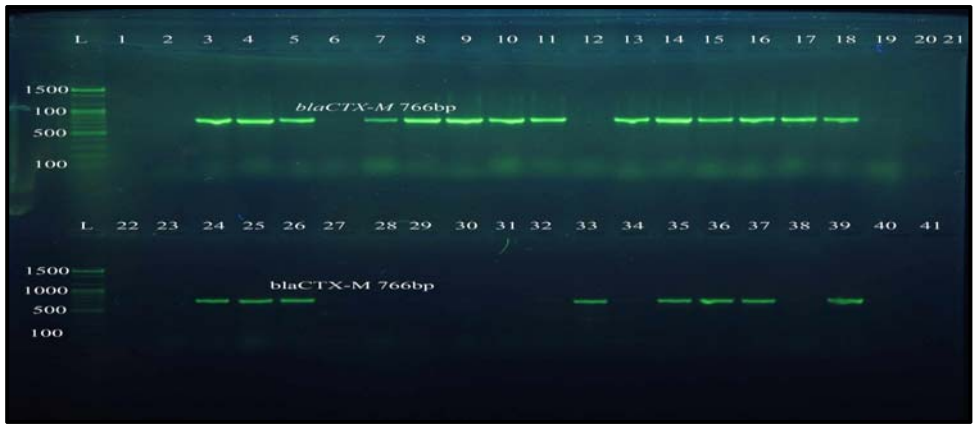

Fig. 7: PCR amplified products from total extracted DNA of 43 *K. pneumoniae* strains. Amplified with *blaCTX-M* gene show positive results at 766 bp
L: DNA molecular size marker

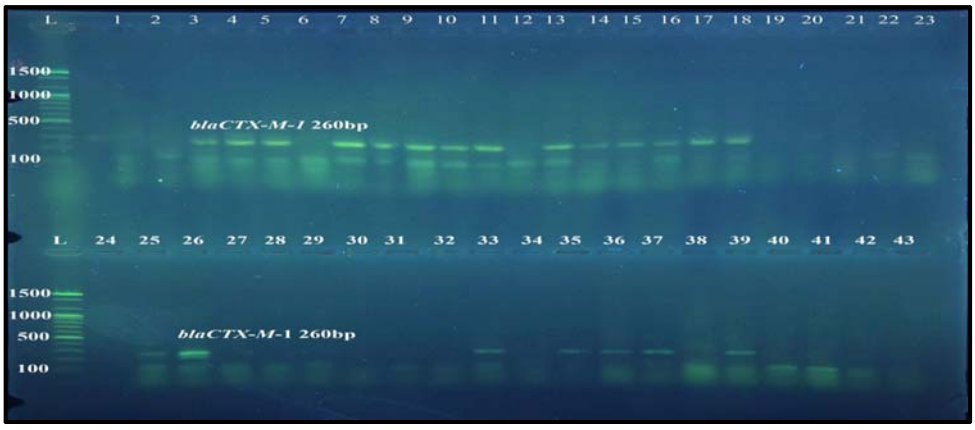

Fig. 8: PCR amplified products from total extracted DNA of 43 *K. pneumoniae*. Amplified with *blaCTX-M-1* gene show positive results at 260 bp
L: DNA molecular size marker

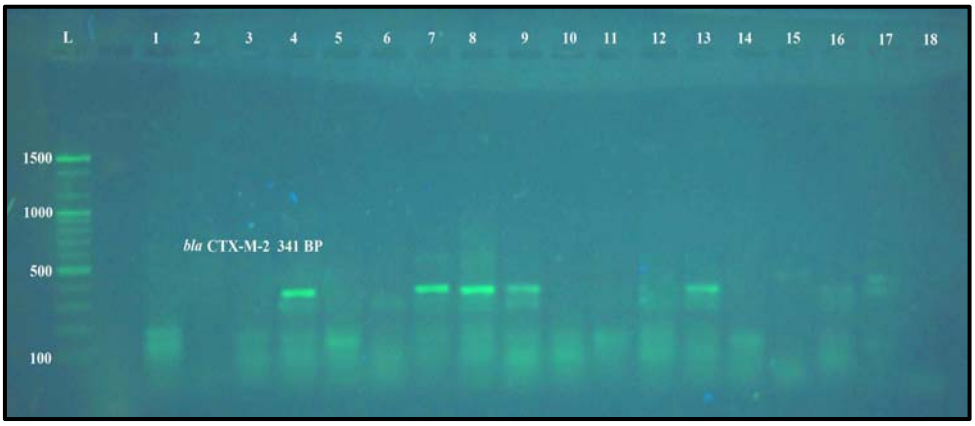

Fig. 9: PCR amplified products from total extracted DNA of 43 *K. pneumoniae* strains. Amplified with *blaCTX-M-2* gene show positive results at 341 bp
L: DNA molecular size marker

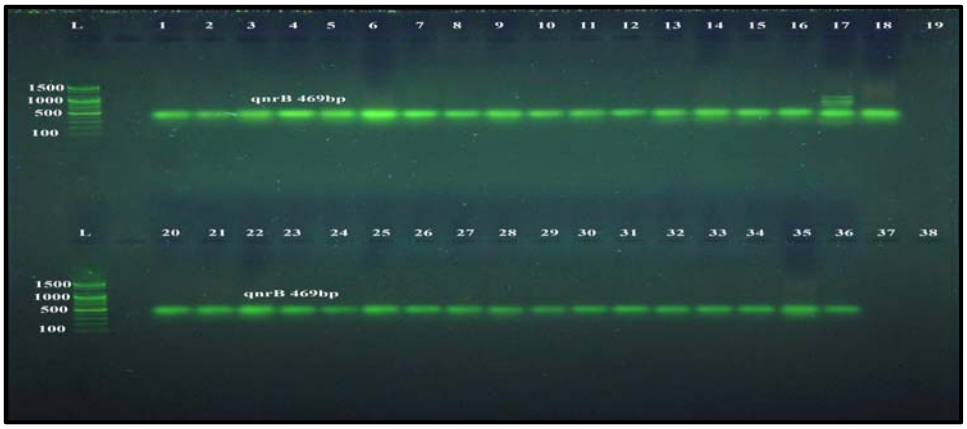

Fig. 10: PCR amplified products from total extracted DNA of 43 *K. pneumoniae* strains. Amplified with *qnrB* gene show positive results at 469 bp
L: DNA molecular size marker

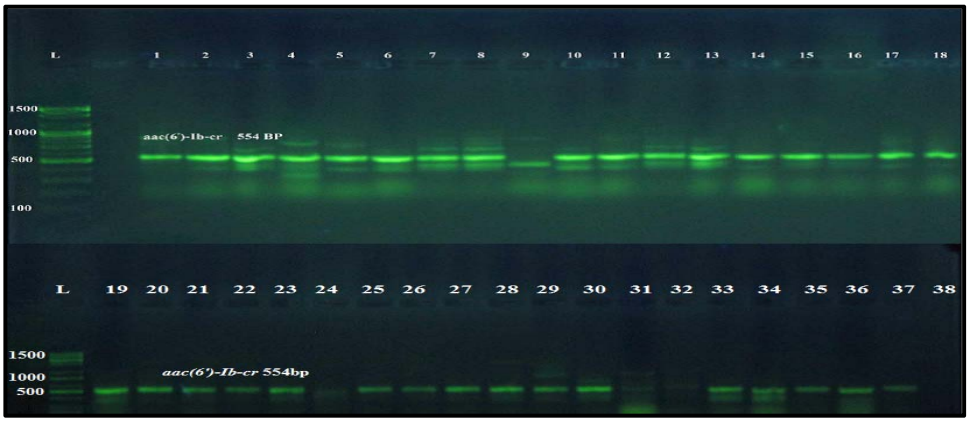

Fig. 11: PCR amplified products from total extracted DNA of 43 *K. pneumoniae* strains. Amplified with *aac(6')-Ib-cr* gene show positive results at 554 bp
L: DNA molecular size marker

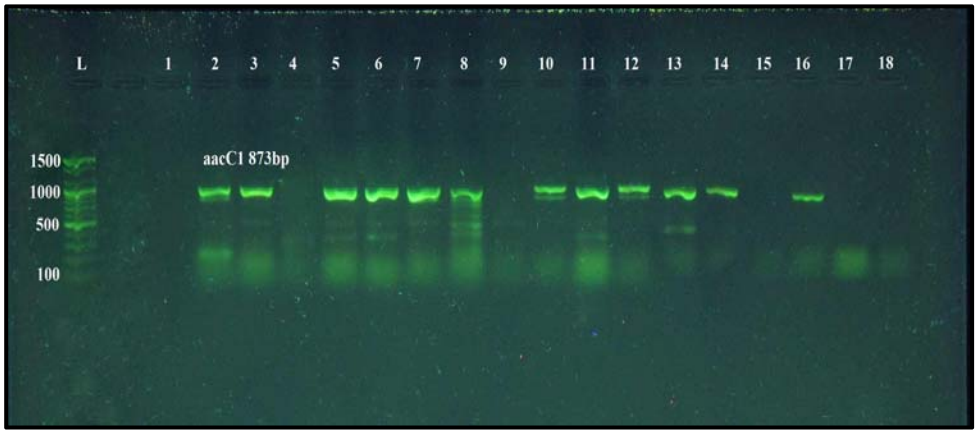

Fig. 12: PCR amplified products from total extracted DNA of 43 *K. pneumoniae* strains. Amplified with *aacC1* gene show positive results at 873 bp
L: DNA molecular size marker

Fig. 13: PCR amplified products from total extracted DNA of 43 *K. pneumoniae* strains. Amplified with *aacC2* gene show positive results at 861 bp
L: DNA molecular size marker

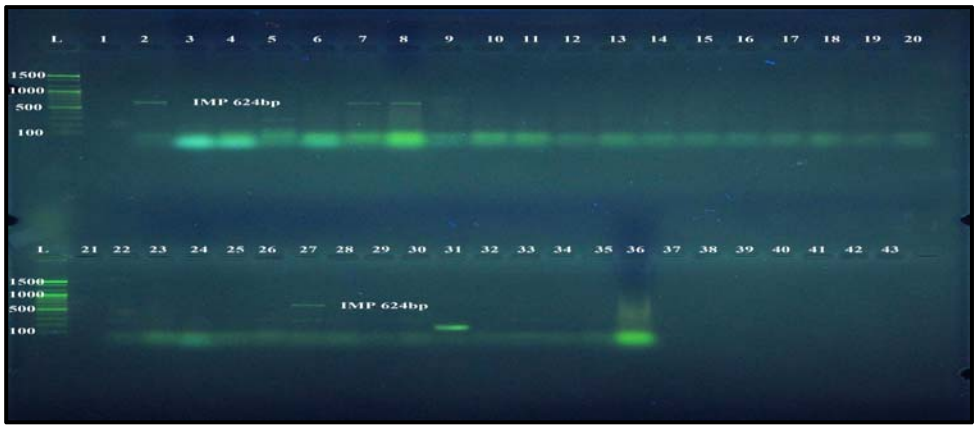

Fig. 14: PCR amplified products from total extracted DNA of 43 *K. pneumoniae*. Amplified with IMP gene show positive results at 624 bp
L: DNA molecular size marker

Table 8: Total and percentages of antimicrobials resistance genes among *K. pneumoniae* strains isolated from in-patients with urinary tract infections and burn infections

	Source of strain		
Genes	Burns 19 strains (100%)	Urine 24 strains (100%)	Total (100%)
blaTEM	13 (68.42)	11 (45.83)	24 (55.81)
blaSHV	19 (100)	18 (75)	37 (86.04)
blaCTX-M	14 (73.68)	8 (33.33)	22 (51.16)
blaCTX-M-1 group	14 (73.68)	7 (29.16)	21 (48.83)
blaCTX-M-2 group	5 (26.31)	0 (0.0)	5 (11.62)
blaCTX-M-8 group	0 (0.0)	0 (0.0)	0 (0.0)
blaCTX-M-9 group	0 (0.0)	0 (0.0)	0 (0.0)
blaCTX-M-25 group	0 (0.0)	0 (0.0)	0 (0.0)
qnrA	0 (0.0)	0 (0.0)	0 (0.0)
qnrB	19 (100)	17 (70.83)	36 (83.72)
aac(6')-Ib-cr	18 (94.73)	15 (62.5)	33 (76.74)
aacC1	12 (63.15)	0 (0.0)	12 (27.90)
aacC2	4 (21.05)	0 (0.0)	4 (9.30)
IMP	3 (15.78)	1 (4.16)	4 (9.30)

Table 9: Genotypic resistance profile of 43 *K. pneumoniae* strains isolated from in-patients infected with urinary tract infections and burn infections

Source of strain	Genotypic resistance profiles	No. (%)
Burns	*blaSHV,qnrB, aac(6')-Ib-cr*	3 (21.42)
Burns	*blaTEM, blaSHV, qnrB, aac(6')-Ib-cr, aacC1, aacC2,IMP*	7 (50)
Burns	*blaSHV,blaCTX-M, blaCTX-M-1, qnrB, aac(6')-Ib-cr, aacC1, aacC2*	7 (50)
Burns	*blaTEM, blaSHV, blaCTX-M, blaCTX-M-1, blaCTX-M-2, qnrB, aac(6')-Ib-cr, aacC2*	8 (57.14)
Burns	*blaSHV, blaCTX-M, blaCTX-M-1, qnrB, aac(6')-Ib-cr, aacC1*	6 (72.85)
Burns	*blaTEM, blaSHV, qnrB, aac(6')-Ib-cr, aacC1*	5 (35.71)
Burns	*blaTEM, blaSHV, blaCTX-M, blaCTX-M-1, blaCTX-M-2, qnrB, aac(6')-Ib-cr, aacC, IMP*	9 (64.28)
Burns	*blaTEM, blaSHV, blaCTX-M, blaCTX-M-1, blaCTX-M-2, qnrB, aac(6')-Ib-cr, aacC1,IMP*	9 (64.28)
Burns	*blaTEM, blaSHV, blaCTX-M, blaCTX-M-1, blaCTX-M-2, qnrB*	6 (72.85)
Burns	*blaTEM, blaSHV, blaCTX-M, blaCTX-M-1, qnrB, aac(6')-Ib-cr, aacC1*	7 (50)
Burns	*blaSHV, blaCTX-M, blaCTX-M-1, qnrB, aac(6')-Ib-cr, aacC1,*	6 (72.85)
Burns	*blaTEM, blaSHV, qnrB, aac(6')-Ib-cr, aacC1,*	5 (35.71)
Burns	*blaTEM, blaSHV, blaCTX-M, blaCTX-M-1, blaCTX-M-2, qnrB, aac(6')-Ib-cr, aacC1*	8 (57.14)
Burns	*blaTEM, blaSHV, blaCTX-M, blaCTX-M-1, qnrB, aac(6')-Ib-cr, aacC1*	7 (50)
Burns	*blaTEM, blaSHV, blaCTX-M, blaCTX-M-1, qnrB, aac(6')-Ib-cr, aacC2*	7 (50)
Burns	*blaTEM, blaSHV, blaCTX-M, blaCTX-M-1, qnrB, aac(6')-Ib-cr, aacC1*	7 (50)
Burns	*blaTEM, blaSHV, blaCTX-M, blaCTX-M-1, qnrB, aac(6')-Ib-cr*	6 (72.85)
Burns	*blaSHV, blaCTX-M, blaCTX-M-1, qnrB, aac(6')-Ib-cr*	5 (35.71)
Burns	*blaSHV, aac(6')-Ib-cr, aac(6')-Ib-cr*	3 (21.42)
Urine	*blaTEM, blaSHV, qnrB, aac(6')-Ib-cr*	4 (28.57)
Urine	*blaTEM, blaSHV, qnrB, aac(6')-Ib-cr*	4 (28.57)
Urine	*blaTEM, blaSHV, qnrB, aac(6')-Ib-cr*	4 (28.57)
Urine	*blaSHV, qnrB, aac(6')-Ib-cr*	3 (21.42)
Urine	*blaSHV, blaCTX-M, qnrB*	3 (21.42)
Urine	*blaCTX-M, blaCTX-M-1, qnrB, aac(6')-Ib-cr*	4 (28.57)
Urine	*blaTEM, blaSHV, blaCTX-M, blaCTX-M-1, qnrB, aac(6')-Ib-cr*	6 (72.85)
Urine	*blaTEM, blaSHV, qnrB, aac(6')-Ib-cr,IMP*	5 (35.71)
Urine	*blaSHV, qnrB, aac(6')-Ib-cr*	3 (21.42)
Urine	*blaTEM,blaSHV, qnrB, aac(6')-Ib-cr*	4 (28.57)
Urine	*qnrB, aac(6')-Ib-cr*	2 (14.28)
Urine	*blaSHV, qnrB*	2 (14.28)
Urine	*blaSHV, qnrB*	2 (14.28)
Urine	*blaTEM, blaCTX-M, blaCTX-M-1, qnrB, aac(6')-Ib-cr*	5 (35.71)
Urine	*blaTEM, blaSHV, qnrB, aac(6')-Ib-cr*	4 (28.57)
Urine	*blaTEM, blaSHV, blaCTX-M, blaCTX-M-1, qnrB, aac(6')-Ib-cr*	6 (72.85)
Urine	*blaTEM, blaSHV, blaCTX-M, blaCTX-M-1, qnrB, aac(6')-Ib-cr*	6 (72.85)
Urine	*blaTEM, blaSHV, blaCTX-M, blaCTX-M-1*	4 (28.57)
Urine		0 (0.0)
Urine	*blaCTX-M, blaCTX-M-1*	2 (14.28)
Urine		0 (0.0)
Urine		0 (0.0)
Urine	*blaSHV*	1 (7.14)
Urine	*blaSHV*	1 (7.14)

No. (100%): Total numbers (n) and percentage (%) of *K. pneumoniae* strains that were carriers of antimicrobial resistance genes

DISCUSSION

The main goal of this study was to investigate the prevalence of drug-resistance *K. pneumoniae* strains, ESBL-producing *K. pneumoniae* strains and molecular detection of antimicrobial resistance-association genes in *K. pneumoniae* strains isolated from inpatients infected with UTI and burns infections in Iraq.

The antimicrobials susceptibility analysis of 43 *K. pneumoniae* strains showed that most strains were highly resistant to antimicrobials especially against third generation cephalosporins with resistance rate ranged from

97.67-90.69%, nitrofurantoin (30 μg) 95.34% and at lower resistant rate against imipenem (10 μg) 9.30% (Fig. 2, 3).

Also the results proved that most *K. pneumoniae* strains were MDR with percentage 74.41-20.93% were XDR and only 4.65% were PDR (Table 5, 7).

In the present study, burns isolates were the most common ESBL producing strains (100%) followed by UTI samples (66.66%) (Fig. 4, Table 6).

Multi-drug resistant and ESBL producing *K. pneumoniae* were significantly observed ($p<0.05$) in *K. pneumoniae* strains isolated from burns infections and these were significantly associated with the increased resistance of *K. pneumoniae* to β-lactam antimicrobials.

Multi-drug resistant bacteria are often associated with ESBL producing bacteria, that is, resistance to other classes of antimicrobials, such as quinolones and aminoglycosides[29]. Most *K. pneumoniae* strains may be having natural resistance to β-lactam antibiotics (amoxicillin, ampicillin and amoxicillin+clavulanic acid) but not to ESBL antibiotics. The resistance to ESBL could happen class A chromosome β-lactamase *TEM* and *SHV* genes are expressed[30]. These genes are capable of enabling bacteria to resist to different antimicrobial agents including third generation cephalosporins, aminoglycosides and others[31-33].

In Iraq, the prevalence of multi-drug-resistant Enterobacteriaceae and ESBL-producing Gram negative bacteria has been reported by some researchers[34,35].

In other countries, there was increase in drug-resistant and ESBL-producing *K. pneumoniae* such as in Algeria[23], Taiwan[36], Turkey[37] and China[38] ranged between 1.5-47%[39].

Klebsiella pneumoniae is a Gram negative bacterium, non-motile, lactose fermenter and can resistant different types of antimicrobials due to produce several resistance-associated genes like, *SHV*, *TEM* and *CTX-M*. According to Podschun and Ullmann,[40] *K. pneumoniae* can be found in the natural environment such as soil and water and in hospitals environment and can be found in healthy individuals tracts such as, respiratory tract, urinary tract and gastrointestinal tract. *Klebsiella pneumoniae* has emerged as an important cause of hospital acquired infections, especially among patients infected with burns infections, the incidence of infections caused by multidrug-resistant *K. pneumoniae* strains has increased in last years.

In 1983 in Europe, the extended spectrum β-lactamase enzymes were first described in *Serratia marcescens* and *K. pneumoniae* strains[41] while, in 1989 in united states, extended spectrum β-lactamase enzymes were described in *K. pneumoniae* and *Escherichia coli* strains[42].

In this study, the prevalence of high resistance rate of antimicrobials and high prevalence of ESBL producing *K. pneumoniae* strains in burns isolates and UTI samples might be due to the extreme empirical use of common antimicrobials such as, β-Lactam, aminoglycosides and 3rd-generation cephalosporins (cefotaxime, ceftriaxone and ceftazidime) in primary infection. In Iraq, higher rates of MDR and ESBL production among *K. pneumoniae* clinical strains have been reported[43,44]. While, the results of the previous studies proved that there were lower rates of ESBL production in Asian countries such as Japan and South Korea and also in United States[45,46]. This differences in prevalence of high resistance rate of antimicrobials and high prevalence of ESBL producing *K. pneumoniae* strains may be attributable to the differences degrees in virulence strains, antimicrobial stewardship program, geographic difference and infection control practices.

In this study, the molecular analysis of the 43 *K. pneumoniae* strains shows there were significant prevalence in β-lactamase (*TEM, SHV* and *CTX-M*) and quinolone resistance-associated genes (*qnrB and aac(6')-Ib-cr*) (Table 8) . And the results proved that *K. pneumoniae* strains isolated from burns infections were carried resistance genes more than those isolated from UTI (Table 9).

Many researchers are focused on the prevalence of β-lactamase genes worldwide, but in Al-Najaf Governorate-Iraq, this is the first study focusing on the prevalence of beta-lactamase genes (*blaSHV*, *blaTEM* and *blaCTX-M* groups) and others genes in *K. pneumoniae* strains isolated from burns infections and UTI.

Klebsiella pneumoniae can initiate burns and wound infections, UTI and pneumonia. The number of outbreaks involving *K. pneumoniae* strains with ESBL mediated resistance to 3rd generation cephalosporins has been progressively increasing in many countries worldwide[47].

Extended spectrum β-lactamases are hydrolyze enzymes portable on bacterial plasmids can hydrolyze oxyimino-beta lactam antimicrobials, such as 3rd generation cephalosporins[5]. There are another resistance genes carrying in the same bacterial plasmids resist to other antimicrobials like, aminoglycosides, tetracycline, sulfonamides, trimethoprim and trimethoprim. Therefore, most Gram negative bacteria containing these plasmids called multi-drug resistance bacteria[48].

The resistance mechanisms of most Gram negative bacteria such as *K. pneumoniae* include different strategies like, the loss of outer membrane proteins and antimicrobial efflux, the production of carbapenemases and β-lactamases (including plasmid-mediated AmpCs and ESBLs) and the production of biological membrane formation factors[49,50].

Extended spectrum β-lactamases are mainly encoded by plasmids and the predominant β-lactamase that mediate *K. pneumoniae* strains and other gram-negative bacterial resistance to new broad spectrum β-lactam antimicrobials. The *blaCTX-M* type (including *blaCTX-M*-groups) and *blaSHV* type are the major ESBLs phenotype were detected worldwide[51,52]. In recent years, *K. pneumoniae* strains carrying beta-lactamase genes such as *blaCTX-M*-type, *blaTEM* and *blaSHV* and quinolone resistance-associated genes and aminoglycosides resistance-associated genes were isolated from patients in several countries[53-56]. In the present study, most MDR *K. pneumoniae* strains and ESBL producing *K. pneumoniae* strains carried the *blaSHV*, *blaTEM* and *blaCTX-M* genes and there were two *K. pneumoniae* strains isolated from burns infections carried nine genes and were PDR. Some previous studies also reported the prevalence of multiple *blaCTX-M*-type genes in *K. pneumoniae* strains[57,58]. The detection of MDR and ESBL-producing *K. pneumoniae* strains and the coexistence of different ESBL-associated genes in the same strain considered a serious public health.

Quinolones are one of the most antimicrobial agents widely used in clinical medicine as a broad spectrum antimicrobial[59]. Among the 43 *K. pneumoniae* strains, the *qnrB* and *aac(6')-Ib-cr* genes were prevalent at high rates. The results are in agreement with some recent studies[60-62]. Prevalence of *aac (6')-Ib-cr* and *qnr* genes in clinical *K. pneumoniae* strains has been reported in several previous studies[63-65]. We believe that this is the first study which focused on the prevalence of *qnrA, qnrB* and *aac(6')-Ib-cr* genes in *K. pneumoniae* strains isolated from inpatients with UTI and burns infection in Iraq. The *qnrA*, *qnrB* and *aac(6')-Ib-cr* are plasmid mediated resistant genes, can easily dissemination between Gram-negative bacteria and lead to difficult to treatment.

CONCLUSION

β-lactamase genes (*blaTEM, blaSHV* and *blaCTX-M*) and quinolone resistance-associated genes (*qnrB* And *aac(6')-Ib-cr*) were commonly prevalence in *K. pneumoniae* strains isolated from burns infections more than those isolated from UTI and there were positive association between high prevalence of these resistance genes and the increased resistance of *K. pneumoniae* strains to β-lactam and quinolone antimicrobials. On the other hand, phenotypic and genotypic methods are required to detect the presence of different resistance-associated genes production in *K. pneumoniae* strains. Therefore, the researchers must work on more preventive measures to reduce colonization and infection by pathogenic bacteria in hospitals.

STATEMENT OF SIGNIFICANCE

This is the first study focusing on the importance of the prevalence of multi-drug resistance *K. pneumoniae* and extended-spectrum β-lactamases producing *K. pneumoniae* strains in hospital patients in Iraq. In this study proved that *K. pneumoniae* strains became highly drug-resistant especially those isolated from in-patients with burns infections.

ACKNOWLEDGMENT

We are grateful to staff of Medical Laboratory in the hospital for providing the clinical samples.

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