RESEARCH ARTICLE

Correlation between antimicrobial resistance and virulence genes in *Klebsiella pneumoniae* isolates from Egypt

Nasma Maged Elemary[1], Mohamed Mahrous Emara[2], Amin Abd Elhady Tahoun[3], Ramadan Ahmed Eloomany[4]

Abstract

Objective: To genotypically assess the relationship between certain resistance and virulence determinants.

Method: The cross-sectional study was conducted at Kafrelsheikh University, Egypt, from March 2019 to May 2021, and comprised pathological samples, like blood, sputum, urine, vaginal swabs and wound swabs, that had been taken from patients who had never received treatment. The sample were collected from Kafrelsheikh and Mansoura University hospitals, and *Klebsiella pneumoniae* isolates were obtained. Resistance and virulence determinants were tested phenotypically. Uniplex polymerase chain reaction was used to evaluate the presence of several resistance-accompanied genes and virulence genes in the isolates. Disc diffusion method was used to assess the isolates' susceptibility in accordance with the Clinical and Laboratory Standards Institute criteria for identifying diverse resistance patterns.

Results: There were 23 isolates from 16 patients. Of the tested isolates, 22(95.65%) showed drug resistance; 19(82.6%) had multidrug resistance, and 3(13.04%) had extensive drug resistance. There was no case of pan drug resistance. CTX-M-15, NDM, CTX-M-1, VIM-1 and qnr B genes were detected in 14(60.86%), 13(56.5%), 6(26.08%), 6(26.08%) and 6(26.08%) isolates, respectively. Moreover, 6(26.08%) isolates exhibited extended-spectrum β-lactamase producers, and 12(52.17%) of such isolates contained both CTX-M-1 and CTX-M-15 genes, 6 and 33.3% contained CTX-M-1, CTX-M-15 and fox genes. Type 3 fimbriae adhesin mrkD and mucoviscosity regulatory gene uge were found in the tested isolates. However, gene of iron uptake system kfu was found in 8(34.78%) isolates, and increased serum survival protein is and mucoviscosity accompanied gene magA were detected in 3(13.04%) isolates. A direct correlation was found among 5 from 8 *Klebsiella pneumoniae* virulence genes and antimicrobial resistance genes.

Conclusion: There was a direct correlation between the existence of virulence factors and resistance to antimicrobials.

Keywords: Lactamase, *Klebsiella pneumoniae*, Anti-bacterial, Virulence, Bacterial, Genes.

DOI: 10.47391/JPMA.EGY-S4-54

Introduction

Infections of the soft tissues, *pneumonia*, bacteraemia and urinary tract are all caused by *Klebsiella (K.) pneumoniae*, which is viewed as a pathogenic opportunist in the developed countries, causing nosocomial infections.[1] A new spectrum of invasive diseases acquired in the community has emerged over the past 3 decades, particularly as pyogenic liver abscesses.[2] However, multidrug-resistant (MDR) and hyper-virulent *K. pneumoniae* (hvKp) strains have emerged independently.[2]

Genetically mobile components, such as plasmids, which may also include virulence determinants, are mostly to blame for the spread of *K. pneumoniae* resistance. The primary global source of antibiotic resistance is K. *pneumoniae*. The resistance of *K. pneumoniae* to various classes of antimicrobial agents is due to a number of mechanisms, such as the secretion of enzymes that break down antimicrobials, modifications to target sites, changes in membrane permeability, efflux pump system activation, and modifications to metabolic pathways. The mechanisms for enzymatic degradation and efflux pump stand out as having a significant impact on the emergence of MDR in *K. pneumoniae*. Additionally, *K. pneumoniae* strains produce harmful enzymes, such carbapenemases, metallo-beta(β)-lactamases (MBLs), oxacillinases and extended spectrum β-lactamases (ESBLs), which can hydrolyse β-lactam antibiotics. The resistance-nodulation division's efflux pumps can expel amphiphilic and charged antimicrobial substances, such β-lactams, aminoglycosides and fluoroquinolones.

Different strains have acquired different levels of virulence, antimicrobial resistance and phylogenetic background. The current study was planned to genotypically assess the relationship between certain resistance and virulence determinants.

Materials and Methods

The cross-sectional study was conducted at Kafrelsheikh

[1]Department of Microbiology and Immunology, Kafrelsheikh University, Egypt.
[2]Department of Microbiology and Immunology, Helwan University, Egypt.
[3]Department of Animal Medicine, Kafrelsheikh University, Egypt.
[4]Department of Animal Pathology, Kafrelsheikh University, Egypt.
Corrospondience: Nasma Maged Elemary
email: nasmamaged58@gmail.com

University, Egypt, from March 2019 to May 2021. After approval form the institutional ethics review committee, pathological samples were collected from Kafrelsheikh and Mansoura University hospitals. These included blood, sputum, urine, vaginal swabs and wound swabs from patients who had never received treatment. The samples found infected with *K. pneumonia* were included, while the rest were excluded. The *K. pneumonia* isolates were subjected to uniplex polymerase chain reaction (PCR), and the identification was confirmed via Vitek2 system (BioMėrieux®, Egypt).

Antibiotic resistance profiles were generated for all the *K. pneumonia* clinical isolates, using Kirby-Bauer disk diffusion (KBDD) in line with the Clinical and Laboratory Standards Institute (CLSI) criteria.[3] Every sample was tested against 16 antibiotics; ampicillin (AMP), ampicillin / sulbactam (SAM), cefazolin (CZ), ceftriazone (CRO), cefepime (FEP), imipenem (IMP), meropenem (MEM), amikacin (AMK), gentamicin (GN), tobramycin (TOB), ciprofloxacin (CIP), nitrofurantoin (FT), trimethoprim / sulfamethoxazole (SXT), tigecycline (TGC), moxifloxacin (MXF), and aztreonam (ATM).

The capsule formation was tested by staining with nigrosine, as per literature,.[4] Haemagglutination was tested based on the slide method by using bacterial fimbriae to detect erythrocytes clumping.[5] The production of biofilm by the isolates of *K. pneumoniae* was tested by following the tube approach.[6] All isolates were checked for the generation of ESBLs using Vitek2 system (BioMėrieux®, Egypt).

Genomic deoxyribonucleic acid (DNA) was extracted for which bacterial colonies were collected and suspended in 100 µL distilled water. The samples were boiled for 15 min, then centrifuged at 5,000 rpm to remove the cell debris. The obtained supernatant was employed as DNA templates.

Uniplex PCR was used to assess the presence of several resistance-accompanying genes in the isolates. The genes tested included ESBLs (blaFOX, CTX-M-1, CTX-M-15), carbapenemases (imipenmase [IMP-1], VIM-1 (Verona Integron-encoded Metallo-β-lactamase), NDM (New delhi Metallo- β- lactamase), KPC (*Klebsiella pneumoniae* carbapenemase), OXA-48 (oxacillinase)), genes of quinolones resistance [qnrA (quinolone resistance gene A), qnrB (quinolone resistance gene B), aac(6)-Ib-cr, and qepA], genes of aminoglycosides resistance (aacA4 and aacC1), genes of cephalosporins resistance (bla dHA) and genes of colistin resistance (mcr1 and mcr2) (mobilized colistin resistance 1,2). PCR was performed using the conditions of specific cycling and primers[7-18] (Table 1).

K. pneumoniae isolates were tested for which PCR was directed at the genes of the two outer membrane porins ompK-35 and ompK36 in line with literature.[19] Conditions used for PCR were initial denaturation at 93 ^{0}C for 3 min, followed by 35 cycles at 93 ^{0}C for 1min, annealing at 55 ^{0}C for 1 min), extension at 72 ^{0}C for 1min and final extension at 72 ^{0}C for 7 min. The amplicons' nucleotide sequences utilised were:

ompK-35 F 5'-ATGATGAAGC GCAATATTCTGGCAGTGG-3',

ompK-35 R 5'-TCGGCTTTGTCGCCA-TTGCCGTCA-3';

OmpK-36 F5'-GAAATTTATAACAAAGACGGC-3',

OmpK-36 R 5'-GACGTTACGTCGTATACTACG-3'.

Uniplex PCR assays for amplification virulence genes included mucoviscosity accompanied gene (magA), gene of DNA gyrase subunit A (gyrA), gene of uridine diphosphate galacturonate 4-epimerase (uge, gene of iron uptake system (kfu), type 1 fimbriae gene (fimH), type 3 fimbriae adhesion gene (mrkD), iron regulatory protein gene (irp2), and increased serum survival protein (iss). Samples of DNA from these genes were amplified using primers and specific cycles[7, 20-26] (Table 2).

Results

Figure 1(A, B, C and D) displays the Antibiotic resistance, Extended-spectrum β-lactamases (ESBLs), Resistance gene expression and Virulence gene expression in *Klebsiella (K.) pneumoniae* isolates. There were 23 isolates from 16 patients. Of the tested isolates, 22(95.7%) showed drug resistance; 19(82.6%) had MDR, and 3(13.04%) had extensive drug resistance (XDR), while there was no case of pan drug resistance (PDR) (Table 3, Figure 1A).

Erythrocyte clumping was found in 21(87.5%) isolates. Biofilm formation was seen in 23(95.8%) isolates (Figure 2).

Further, 6(25%) isolates were ESBL producers, and 18(75%) were non-producers (Figure 1B). The association between ESBL generation and antibiotic resistance was noted (Figure 3).

The β-lactamase genes in a selected isolate, identified using PCR, predicted that sizes 873, 955, 190, 261, 95, 1000 and 744 bp were correlated to CTX-M-1, CTX-M-15, FOX, VIM, KPC, NDM, and OXA-48, respectively. CTX-M-1 gene was observed in 6(26.08%) isolates, and CTX-M-15 in 14(60.86). FOX and NDM genes were observed in 10(43.47%) and 13(56.5%) isolates, respectively. OXA-48 was found in 4(17.39%) isolates. VIM-1 was observed in 6(26.08%) isolates. KPC and dHA genes were not observed in any isolates. CTX-M-1 and CTX-M-15 were observed in 16(69.56%) and 23(100%), respectively. ESBL-producing

isolate's FOX gene was present in 12(52.17%) of them. However, 11(47.8%) of non-producers contained CTX-M-15, 3(13.04%) harboured CTX-M-1, while 6 (33.3%) of the non-producers harboured FOX gene. OXA-48 was detected in 8(34.78%) of ESBL isolates, whereas it was observed in 3(13.04%) of the non-producers. VIM gene was not detected at all in ESBL isolates, while it was found in 8(34.78%) of the non-producers. NDM gene was found in 18(78.26%) of the non- producers, but not in ESBL isolates.

For qnrA, qnrB, aac(6')-Ib-cr, and qepA, respectively, the visualisation of amplified products was done with approximately 572, 469, 482 and 403 bp in size. A total of 2 isolates contained qnrA, whereas 6 isolates harboured qnrB. Additionally, 10 isolates exhibited aac(6´)-Ib-cr gene, and no isolate harboured qepA.

The aacC1 and aacC4 genes' corresponding amplified products had sizes of 456 and 518bp, respectively. Only 2 isolates tested positive for the aacC1 gene, while 8 isolates had

Table-1: Antibacterial resistance profiles of Klebsiella (K.) pneumonia isolates and their sources.

Bacterial Isolate	Source	AMP	AMP /SAM	CZ	CRO	FEP	MEM	AMK	GN	TOB	CIP	FT	SXT	IMP	TGC	MXF	ATM	ESBL	MDR or XDR	Resistance genes	Virulence genes
935	Blood	R ≥32	R ≥32	R≥64	R ≥64	R ≥64	R≥16	R≥64	R≥16	R≥16	R ≥4	R ≥512	R ≥320	R ≥16	S≤0.5	R ≥8	R ≥64	-ve	XDR	NDM, CTX-M 15, aacC4, aacC6	Uge, Iss, gyr A, mrk D
755	Blood	R=16	S=4	S ≤4	S≤1	S ≤1	S≤0.25	S ≤2		S ≤1	S≤0.25	I=64	S≤20	S≤0.25	S≤0.5	S ≤0.25	S ≤1	-ve	-	No	Kfu, mrkD
23	Blood	R ≥32	R ≥32	R ≥64	R≥64	R ≥64	R≥16	R≥64	R≥16	R ≥16	R ≥4	R =256	R ≥320	I=2	S≤0.5	R≥8	R≥64	-ve	XDR	Vim1, NDM, aacC1, aacC4, aacC6, OmpK35	Uge, mrkD
71	Urine	R ≥32	R ≥32	R≥64	R≥64	S=8	R=4	S=4	S≤1	I=8	R≤0.25	R≤16	R≤20	S=1	S≤0.5	R=0.5	R≥64	-ve	MDR	NDM, bla Fox	Uge, Kfu, mrkD
47	Blood	R ≥32	R ≥32	R ≥64	R≥64	R ≥64	S≤0.25	R≥64	R≥16	R ≥16	R ≥4	R=256	R=40	S =0.5	S=1	R ≥8	R≥64	-ve	MDR	CTX-M-15, CTX-M-1, aacC4,, aacC6	Uge, mrkD
788	Blood	R ≥32	I=16	R≥64	R≥64	R=2	S≤0.25	S≤2	S≤1	S≤1	S=0.5	S=32	S≤20	S≤0.25	S≤0.5	S=2	R=16	+ve	MDR	OXA-48, CTX-M-15, bla fox, OmpK35, OmpK36	Uge, Kfu, mrkD
700	Blood	R≥32	R≥32	R≥64	R≥64	R≥64	S≤0.25	S=4	R≥16	R≥16	R≥4	S=32	R≥320	S≤0.25	S≤0.5	R≥8	R≥64	-ve	MDR	OXA-48.QnrB, CTX-15, aacC4	Uge, gyrA, mrkD
80	Urine	R≥788	R≥32	R≥64	R≥64	R*16	R≥16	S=16	S≤1	R≥16	S≤0.25	S≤16	R≥320	R≥16	S=1	S≤0.25	R*≤1	-ve	MDR	NDM,	Uge, Kfu, mrkD
799	Blood	R≥32	R≥32	R≥64	R≥64	R≥64	R≥16	S=4	S≤1	R≥16	R≥4	R≥512	R≥320	R≥16	S=2	R≥8	R≥64	-ve	MDR	Vim1, QnrB, NDM, CTX-M-15, bla fox, OmpK35, OmpK36	Uge, Iss, gyrA, Kfu, mrkD
12	Blood	R≥32	I=16	R≥64	R≥64	R*2	S≤0.25	S≤2	S≤1	S≤1	R≥4	S=32	R≥320	R≤0.25	S=1	R≥8	R=16	+ve	MDR	QnrB, CTX-M-15, aacC6, OmpK36	Uge, mrkD
25	Blood	R≥32	R≥32	R≥64	R≥64	R≥64	R≥16	S=16	S≤1	R≥16	R≥4	R≥512	R≥320	R≥16	S=2	R≥8	R≥64	-ve	MDR	Vim1,NDM, CTX-M-15	magA,Uge, Iss, mrkD
1682	Blood	R≥32	R≥32	R≥64	R≥64	S=2	S≤0.25	S≤2	S≤1	I=8	R≥4	R=256	R≥320	S≤0.25	R=8	R≥8	R≥64	+ve	MDR	OXA-48,QnrB, CTX-M-15, CTX-M-1, bla fox, OmpK36	Uge, Kfu, mrkD
107	Blood	R≥32	R≥32	R≥64	R≥64	R≥64	R≥16	S=16	R≥16	R≥16	S=1	S≤16	S=40	R≥16	S=1	S=2	R≥64	-ve	MDR	NDM, CTX-M-15, aacC1, aacC6, OmpK36	Uge, mrkD
84	Urine	R≥32	R≥32	R≥64	R≥64	R≥64	R≥16	S≤2	S≤1	S≤1	R≥4	R≥512	S=40	R≥16	S≤0.5	R≥8	R≥64	-ve	MDR	QnrB, NDM, CTX-M-15, bla Fox, aacC4, aacC6, OmpK36	Uge, Kfu, mrkD
63	Urine	R≥32	R≥32	R≥64	R≥64	R*16	R=4	R≥64	R≥16	R≥16	R≥4	R=256	R≥320	R≥16	S=2	R≥8	R*≤1	-ve	XDR	Vim1, NDM, aacC6	magA, Uge, mrkD
86	Sputum	R≥32	R≥32	R≥64	R≥64	R≥64	R≥16	S=16	R≥16	R≥16	I=2	S≤16	S≤20	R≥16	S≤0.5	S=2	R≥64	-ve	MDR	QnrA, OXA-48, CTX-M-15, bla Fox, aacC4, aacC6, OmpK36	Uge, Kfu, mrkD
89	Blood	R≥32	R≥32	I=16	S≤1	S≤1	S≤0.25	S≤2	S≤1	S≤1	S≤0.25	S≤16	R≥320	S≤0.25	S≤0.5	S≤0.25	S≤1	-ve	MDR	bla fox, aacC4	FimH, Uge, mrkD
55	Vaginal tube	R≥32	R≥32	S≤4	S≤1	S≤1	S≤0.25	S=8	S≤1	S≤1	S≤0.25	S=32	S≤20	S≤0.25	S≤0.5	S≤0.25	S=2	-ve	-	OmpK36	Uge, gyrA, mrkD
929	Urine	R≥32	R≥32	R≥64	R≥64	R≥64	R≥16	S=16	S≤1	R≥16	R≥4	R≥512	S≤20	R≥16	I=4	R≥8	R≥64	-ve	MDR	Vim1, NDM	Uge, mrkD
41	Vaginal tube	R≥32	R≥32	R≥64	R≥64	R≥64	R≥16	S≤2	S≤1	S≤1	R≥4	S=32	R≥320	R*1	S≤0.5	R≥8	R*4	-ve	MDR	QnrA, Vim1, CTX-M-1, bla fox, aacC4, aacC6	magA, Uge, FimH,mrkD
68	Urine	R* 16	S=4	S≤4	S≤1	S≤1	S≤0.25	S≤2	S≤1	S≤1	R≥4	R=256	R≥320	S≤0.25	S=2	R≥8	S≤1	-ve	MDR		gyrA, mrkD
Ui'42	Vaginal tube	R≥32	I=16	R≥64	R≥64	R*=2	S≤0.25	S≤2	S≤1	S≤1	S=1	I=64	R≥320	S≤0.25	S≤0.5	S=2	R=16	+ve	MDR	CTX-M-15, CTX-M-1, bla fox, OmpK35, OmpK36	
59	Vaginal tube	R≥32	I=16	R≥64	R≥64	R*=2	S≤0.25	S≤2	S≤1	S≤1	S=1	S=32	R≥320	S≤0.25	S=1	S=2	R=16	+ve	MDR	CTX-M-15, CTX-M-1, OmpK36	Uge, mrkD

AMP: Ampicillin, AMP/SAM: Ampicillin/Sulbactam, CZ: Cefazoline, CRO: Ceftriaxone, FEP: Cefepime, MEM: Meropenem, AMK: Amikacin, GN: Gentamicin, TOB: Tobramycin, CIP: Ciprofloxacin, FT: Nitrofurantoin, SZT: Trimethoprim /Sulfamethoxazole, IMP: Imipenem, TGC: Tigecycline, MXF: Moxifloxacin, ATM: Aztreonam, ESBL: Extended-spectrum β-lactamase, MDR: Multidrug resistance, XDR: Extensive drug resistance.

Table-2: Reaction cycling conditions for the antimicrobial resistance genes of Klebsiella (K.) pneumonia.

Primers	Sequences (5′–3′)	Target gene	Initial Denaturation	Denaturation	Annealing	Extension	Final Extension	Size of amplified product (bp)	Ref.
fox F	AACATGGGGTATCAGGGAGATG	bla fox	94 °C, 5min	94 °C, 1min	52.5 °C, 30 sec	72 °C, 1 min	72 °C, 10 min	190	6
fox R	CAAAGCGCGTAACCGGATTGG			30 Cycles					
dHA F	AACTTTCACAGGTGTGCTGGGT	bla dHA	94 °C, 5min	94 °C, 1min	53.5 °C, 30 sec	72 °C, 1 min	72 °C, 10 min	405	6
dHA R	CCGTACGCATACTGGCTTTGC			30 Cycles					
CTX-M-1F	ATGTGCAGYACCAGTAARGT	bla CTX-M-1	94 °C, 5min	94 °C, 1min	49 °C, 30 sec	72 °C, 1 min	72 °C, 10 min	873	7
CTX-M-1R	TGGGTRAARTARGTSACCAGA			30 Cycles					
CTX-M-15F	CACACGTGGAATTTAGGGACT	bla CTX-M-15	94 °C, 5min	94 °C, 1min	49.5 °C, 40 sec	72 °C, 1 min	72 °C, 10 min	995	8
CTX-M-15R	GCCGTCTAAGGCGATAAACA			30 Cycles					
oxa48F	TTGGTGGCATCGATTATCGG	bla OXA 48	94 °C, 5min	94 °C, 1min	56 °C, 40 sec	72 °C, 1 min	72 °C, 10 min	744	9
oxa48R	GAGCACTTCTTTTGTGATGGC			30 Cycles					
vim1F	AGTGGTGAGTATCCGACAG	bla VIM1	94 °C, 5min	94 °C, 1min	48 °C, 40 sec	72 °C, 1 min	72 °C, 10 min	261	10
vim1R	ATGAAAGTGCGTGGAGAC			30 Cycles					
ndmF	ATGAAAGTGCGTGGAGAC	bla NDM	94 °C, 5min	94 °C, 1min	47.5 °C, 40 sec	72 °C, 1 min	72 °C, 10 min	1000	11
ndm R	TCGCATAAAACGCCTCTG			30 Cycles					
qnrAF	GAAACTGTCGCACCTCAT	qnrA	94 °C, 5min	94 °C, 1min	57 °C, 1 min	72 °C, 1 min	72 °C, 10 min	572	12
qnrA R	TTCTCACGCCAGGATTTGAG			30 Cycles					
qnrB F	GATCGTGAAAGCCAGAAAGG		94 °C, 5min	94 °C, 1min	50.5°C, 1min	72°c, 1 min	72 °C, 10 min	469	13
qnrB R	ACGATGCCTGGTAGTTGTCC	qnrB		30 cycles					
qepA F	CTGCAGGTACTGCGTCATG		94 °C, 5min	94 °C, 1min	50 °C, 40 sec	72 °C, 1 min	72 °C, 10 min	403	12
qepA R	CGTGTTGCTGGAGTTCTTC	qepA		30 Cycles					
aac(6)-Ib-cr F	TTGCGATGCTCTATGAGTGGCTA		94 °C, 5min	94 °C, 1min	53 °C, 40 sec	72 °C, 1 min	72 °C, 10 min	482	14
aac(6)-Ib-cr R	CTCGAATGCCTGGCGTGTTT	aac(6)-Ib-cr		30 Cycles					
aacC1 F	ATGGGCATCATTCGCACATGTAGG		94 °C, 5min	94 °C, 1min	54.5 °C, 40 sec	72 °C, 1 min	72 °C, 10 min	456	15
aacC1 R	TTAGGTGGCGGTACTTGGGTC	aacC1		30 Cycles					
aacC4 F	ATGACTGAGCATGACCTTGCG		94 °C, 1min	94 °C, 1min	52.5 °C, 40 sec	72 °C, 1 min	72 °C, 10 min	518	15
aacC4 R	TTAGGCATCACTGCGTGTTCG	aacC4		30 Cycles					
mcr1 F	ATCAGCCAAACCTATCCCATCG		94 °C, 1min	94 °C, 1min	52.5 °C, 40 sec	72 °C, 1 min	72 °C, 10 min	1257	16
mcr1 R	GCAGACGCACAGCAATGCCTAT	mcr1		30 Cycles					
mcr2 F	GCGATGGCGGTCTATCCTGTAT		94 °C, 5min	94 °C, 1min	55 °C, 1min	72 °C, 1 min	72 °C, 10 min	378	16
mcr2 R	TGCGATGACATGGGGTGTCAGC	mcr2		30 Cycles					
kpc F	ATATCTGACAACAGGCATGAC		94 °C, 5min	94 °C, 1min	48 °C, 1min	72 °C, 1 min	72 °C, 10 min	95	17
kpc R	CCAACTCCTTCAGCAACAAA	Kpc		30 Cycles					

Table-3: Klebsiella (K.) pneumoniae virulence genes cyclic conditions for polymerase chain reaction (PCR).

Primers	Sequences (5′–3′)	Target gene	Size of amplified product (bp)	Initial Denaturation	Denaturation	Annealing	Extension	Fina Extension	Ref.
irp2 F	ATTTCTGGCGCACCATCT	Irp2	954	94 °C, 2 min	94 °C, 1 min	65 °C, 2 min	72 °C, 3 min	72 °C, 7 min	19
irp2 R	GCGCCGGGTATTACGGACTTC			30 cycles					
magA F	CGCCGCAAATACGAGAAGTG	magA	1283	94 °C, 5 min	94 °C, 1 min	50.5 °C, 40 sec	72 °C, 1 min	72 °C, 10 min	20
magA R	GCAATCGAAGTGAAGAGTGC			30 cycles					
fimH F	CGAGTTATTACCCTGTTTGCTG	fimH	674	94 °C, 5 min	94 °C, 1 min	48.5 °C, 40 sec	72 °C, 1 min	72 °C, 10 min	21
fimH R	ACGCCAATAATCGATTGCAC			30 Cycles					
uge F	GATCATCCGGTCTCCCTGTA	Uge	534	94 °C, 5 min	94 °C, 1 min	51 °C, 40 sec	72 °C, 1 min	72 °C, 10 min	22
uge R	TCTTCACGCCTTCCTTCACT			30 cycles					
iss F	TGTCACATAGGATTCTGCCGTT	iss	455	94 °C, 5 min	94 °C, 1 min	52 °C, 40 sec	72 °C, 1 min	72 °C, 10 min	6
iss R	TTCACCCTCAGAGAGAGGCT			30 cycles					
gyrA F	TGCCAGAGAAATTACACC	gyrA	3072	94 °C, 5 min	94 °C, 1 min	44 °C, 40 sec	72 °C, 1 min	72 °C, 10 min	23
gyrA R	AATATGTTCCATCAGCCC			30 Cycles					
Kfu F	GGCCTTTGTCCAGAGCTACG	kfu	638	94 °C, 5 min	94 °C, 1 min	54 °C, 1 min	72 °C, 1 min	72 °C, 10 min	24
Kfu R	GGGTCTGGCGCAGAGTATGC			30 Cycles					
mrkD F	AAGCTATCGCTGTACTTCCGGCA	mrkD	340	94 °C, 5 min	94 °C, 1 min	55.5 °C, 1 min	72 °C, 1 min	72 °C, 10 min	25
mrkD R	GGCGTTGGCGCTCAGATAGG			30 Cycles					

the aacC4 gene. Additionally, 1 isolate contained the aacC1 and aacC4 genes. No isolates had the mcr1 or mcr2 genes.

There were 4 isolates positive for ompK-35, while 13 tested positive for ompK-36. The ompK-35 was found in 33.3% of ESBL-producing isolates and 11.1% of non-producing isolates. The ompK-36 was found in 83.3% of ESBL isolates and 27.78% of non-producing isolates.

For irp2, magA, fimH, uge, iss, gyrA, kfu and mrkD, the amplicons had approximate sizes were 954, 1283, 674, 534, 455, 3072, 638, and 340 bp, respectively. The irp2 was not detected in any isolate. The iss and magA genes were both detected in 3 isolates. All isolates harboured uge and mrkD genes. There were 5(20.8%) isolates that tested positive for gyrA, 2(8.3%) for fimH and 8(33.3 %) for kfu gene. The isolates that produced ESBLs lacked the iss and magA genes, whereas 16.7% of the non-producers did. The fimH and gyrA genes were absent in ESBL-producing isolates,

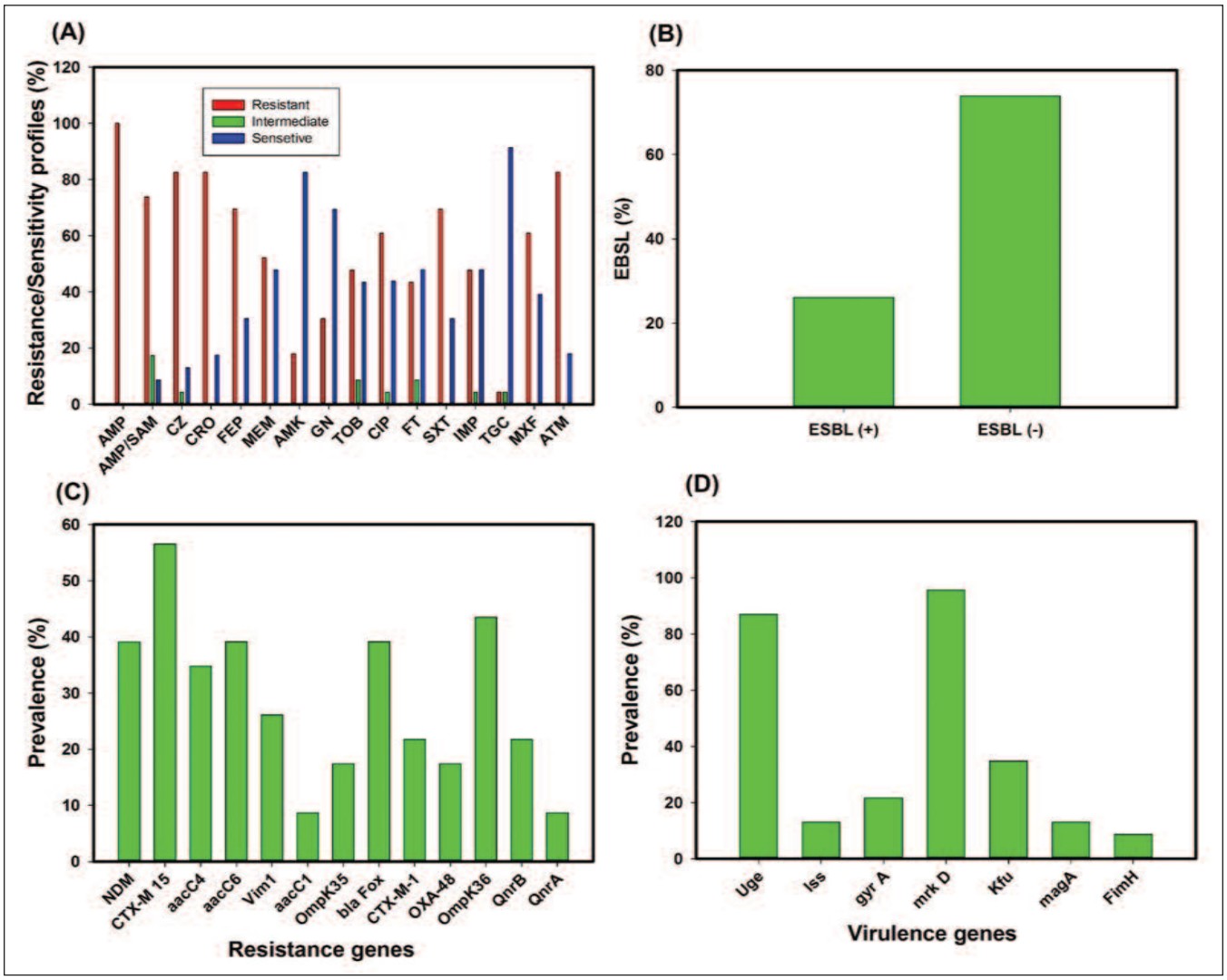

Figure 1: A. Antibiotic resistance in Klebsiella (K.) pneumoniae isolates. B. Extended-spectrum β-lactamases (ESBLs) in K. pneumoniae isolates. C. Resistance genes expressed in K. pneumonia isolates. D. Virulence gene expression in K. pneumoniae isolates.
AMP: Ampicillin, AMP/SAM: Ampicillin/Sulbactam, CZ: Cefazoline, CRO: Ceftriaxone, FEP: Cefepime, MEM: Meropenem, AMK: Amikacin, GN: Gentamicin, TOB: Tobramycin, CIP: Ciprofloxacin, FT: Nitrofurantoin, SZT: Trimethoprim /Sulfamethoxazole, IMP: Imipenem, TGC: Tigecycline, MXF: Moxifloxacin, ATM: Aztreonam.

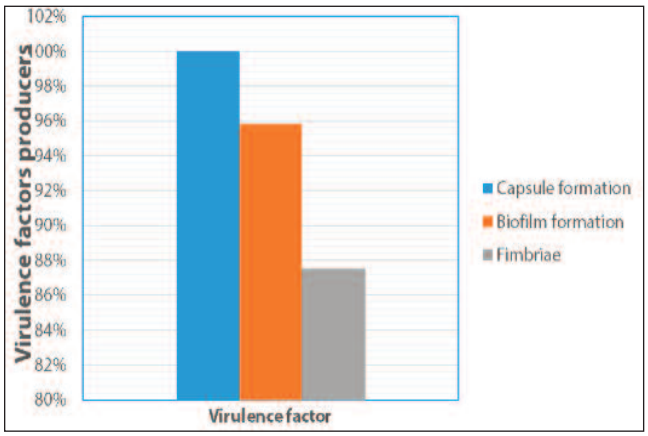

Figure 2: Virulence components in *Klebsiella (K.) pneumonia* isolates.

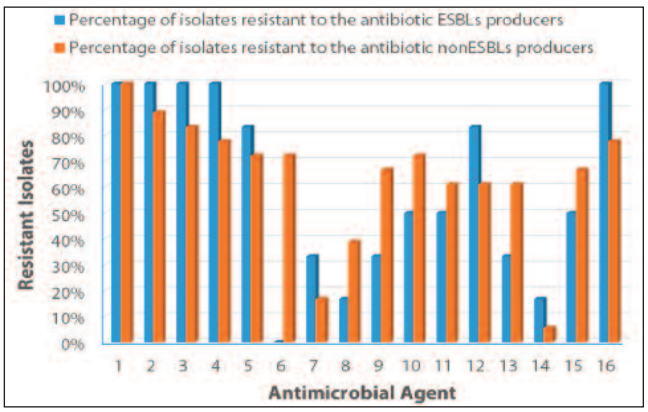

Figure 3: Association between antibiotic resistance and extended-spectrum β-lactamase (ESBL) synthesis in *Klebsiella (K.) pneumonia* isolates

while they were found in 12.5% and 27.8%, for fimH and gyrA, respectively, in the non-producers. The kfu gene was found in 8(33.3 %) of ESBL isolates, and 33.3% of the non-producing isolates (Figure 1C-D).

Discussion

To the best of our knowledge, the current study is the first to discover the development of ESBLs using modified three-dimensional (3D) assays to confirm the production of *K. pneumoniae* MBLs. The findings suggested that MBLs produced by *K. pneumoniae* isolates represented an important mechanism providing cephalosporin and carbapenem resistance.

The current study examined the susceptibility of 23 isolates of *K. pneumoniae* to 16 different antibiotics, and then investigated the connection between the virulence factors and resistance profiles in the isolates. It found MDR profiles for 23 isolates 22(91.7%), of which 19(82.6%) were MDR, 3(13.04%) were XDR, and none was PDR. Among other findings, the current study noted that 33.3% of ESBL-producing isolates included CTX-M-1, CTX-M-15 and FOX genes. The incidence of CTX-M-1 and CTX-M-15 genes have been reported differently earlier.[27] The current isolates lacked obvious IMP and KPC genes the importance of which have been reported by Han., R. et al.[28]

Bahmani N, et al.[29] reported that VIM-1 gene was present in 26.7% isolates, while it was present in 6 (26%) of the current isolates.

In the current study, only 2 (8.69%) isolates contained qnrA gene, which is comparable to the 10% reported by a Chinese study.[29] The qnrB gene, on the other hand, was found in 25% isolates, which was much higher than the Chinese study.[30] Furthermore, the aac(6')-Ib-cr gene was found in 41.7% of the current isolates, which is consistent with other studies.[19] The current isolates had no detectable qepA which is in agreement with Malek Jamshidi, M. R., et al.[31] Interestingly, although 15(65.2 %) isolates showed quinolone resistance, 10 of them expressed quinolone resistance genes (Table 1).

The current study found that 13 (56.5%) isolates were resistant to aminoglycosides, and 5 (21.7%) of them harboured aacC1 or aacC4 genes, while 1 (4.3%) harboured both the genes, which was a novel coexistence.

In addition, 7(29.2 %) isolates had both a CTX-M type β-lactamases and any of aminoglycosides resistance genes. In contrast, Latifi, B., et al.[32] detected relatively higher percentage (68.08%) for the existence of both the genes.

All *K. pneumoniae* isolates underwent phenotypic screening and noted the presence of certain virulence factors, including production of biofilms, fimbriae, and capsules. All the isolates under evaluation were found to be capsuled, which was in line with earlier findings[33]. On the other hand, 22 (95.8%) of the examined samples produced biofilm, and 20 (87.5%) expressed fimbriae. The current study indicated the presence of mrkD gene, whereas 2 (8.69%) of selected isolates indicated the presence of both the fimH and mrkD genes. Higher detection of fimH and mrkD genes have been reported earlier[33]. FimH- and mrkD-containing isolates were found to harbour many resistance genes in the current study, which is in close agreement with reported results[34], showing a substantial relationship between the presence of fimbriae and antibiotic resistance. Kfu, a crucial iron absorption mechanism, was involved in the production of capsules as well as purulent tissue infections. In the current study, the prevalence's of the irp2 and kfu genes among *K. pneumoniae* isolates were different (0% and 8 (34.78%), respectively). In contrast, a study detected higher percentages of these genes[35]. However, uge, which was related to capsule lipoprotein, was observed in 100% of the isolates, which was higher than that reported previously.[36]

On the other hand, magA was found in 3 (13.04 %) of the isolates, which was a higher than that detected earlier.[37] The current results showed that the iss gene was found in 3 isolates from blood source, suggesting it is found in blood. The fimH was found in blood and vaginal tube isolates as the fimbriae is found in the urinary system.

The current study was conducted in the Middle and North Delta Region in Egypt, and, therefore, the bacterial antimicrobial resistance profile may particularly help microbiologists and clinicians in better managing infections in that region.

Conclusion

There was a direct correlation between the existence of virulence factors and resistance to antimicrobials. In view of the clear correlation, it is also possible to predict the factors that determine virulence based on resistance patterns.

Acknowledgement: We are grateful to the doctors and nurses of Kafrelsheikh and Mansoura hospitals for their help in the sampling process.

Disclaimer: The text is based on a PhD thesis.

Conflict of Interest: None.

Source of Funding: Kafrelsheikh University, Egypt.

References

1. Hennequin C, Robin F. Correlation between antimicrobial resistance and virulence in Klebsiella pneumoniae. Eur J Clin Microbiol Infect Dis 2016;35:333-41. doi: 10.1007/s10096-015-2559-7.
2. Chang FY, Chou MY. Comparison of pyogenic liver abscesses caused by Klebsiella pneumoniae and non-K. pneumoniae pathogens. J Formos Med Assoc 1995;94:232-7.
3. Clinical Laboratory Standards Institute. M02: Performance standards for antimicrobial disk susceptibility tests, 13th ed. Wayne, PA: CLSI; 2018.
4. Sørensen UB. Pneumococcal polysaccharide antigens: capsules and C-polysaccharide. An immunochemical study. Dan Med Bull 1995;42:47-53.
5. Vagarali MA, Karadesai SG, Patil CS, Metgud SC, Mutnal MB. Haemagglutination and siderophore production as the urovirulence markers of uropathogenic Escherichia coli. Indian J Med Microbiol 2008;26:68-70. doi: 10.4103/0255-0857.38863.
6. Christensen GD, Simpson WA, Bisno AL, Beachey EH. Adherence of slime-producing strains of Staphylococcus epidermidis to smooth surfaces. Infect Immun 1982;37:318-26. doi: 10.1128/iai.37.1.318-326.1982.
7. Ewa K, Tomasz D, Grażyna M. Rapid detection of NDM, VIM, KPC and IMP carbapenemases by real-time PCR. J Bacteriol Parasitol 2016;7:2-5. Doi: 10.4172/2155-9597.1000299
8. Liu X, Liu Y. Detection of plasmid-mediated AmpC β-lactamase in Escherichia coli. Biomed Rep 2016;4:687-90. doi: 10.3892/ br.2016.661.
9. Mugnaioli C, Luzzaro F, De Luca F, Brigante G, Amicosante G, Rossolini GM. Dissemination of CTX-M-type extended-spectrum beta-lactamase genes to unusual hosts. J Clin Microbiol 2005;43:4183-5. doi: 10.1128/JCM.43.8.4183-4185.2005.
10. Muzaheed, Doi Y, Adams-Haduch JM, Endimiani A, Sidjabat HE, Gaddad SM, et al. High prevalence of CTX-M-15-producing Klebsiella pneumoniae among inpatients and outpatients with urinary tract infection in Southern India. J Antimicrob Chemother 2008;61:1393-4. doi: 10.1093/jac/dkn109.
11. Brink AJ, Coetzee J, Corcoran C, Clay CG, Hari-Makkan D, Jacobson RK, et al. Emergence of OXA-48 and OXA-181 carbapenemases among Enterobacteriaceae in South Africa and evidence of in vivo selection of colistin resistance as a consequence of selective decontamination of the gastrointestinal tract. J Clin Microbiol 2013;51:369-72. doi: 10.1128/JCM.02234-12.
12. Giakkoupi P, Xanthaki A, Kanelopoulou M, Vlahaki A, Miriagou V, Kontou S, et al. VIM-1 Metallo-beta-lactamase-producing Klebsiella pneumoniae strains in Greek hospitals. J Clin Microbiol 2003;41:3893-6. doi: 10.1128/JCM.41.8.3893-3896.2003
13. Shen Y, Xiao WQ, Gong JM, Pan J, Xu QX. Detection of New Delhi Metallo-Beta-Lactamase (Encoded by blaNDM-1) in Enterobacter aerogenes in China. J Clin Lab Anal 2017;31:e22044. doi: 10.1002/jcla.22044.
14. Cattoir V, Poirel L, Nordmann P. Plasmid-mediated quinolone resistance pump QepA2 in an Escherichia coli isolate from France. Antimicrob Agents Chemother 2008;52:3801-4. doi: 10.1128/AAC.00638-08.
15. Wang A, Yang Y, Lu Q, Wang Y, Chen Y, Deng L, et al. Presence of qnr gene in Escherichia coli and Klebsiella pneumoniae resistant to ciprofloxacin isolated from pediatric patients in China. BMC Infect Dis 2008;8:68. doi: 10.1186/1471-2334-8-68.
16. Hujer KM, Hujer AM, Hulten EA, Bajaksouzian S, Adams JM, Donskey CJ, et al. Analysis of antibiotic resistance genes in multidrug-resistant Acinetobacter sp. isolates from military and civilian patients treated at the Walter Reed Army Medical Center. Antimicrob Agents Chemother 2006;50:4114-23. doi: 10.1128/AAC.00778-06.
17. Yang F, Shen C, Zheng X, Liu Y, El-Sayed Ahmed MAE, Zhao Z, et al. Plasmid-mediated colistin resistance gene mcr-1 in Escherichia coli and Klebsiella pneumoniae isolated from market retail fruits in Guangzhou, China. Infect Drug Resist 2019;12:385-9. doi: 10.2147/IDR.S194635.
18. Doma AO, Popescu R, Mituleţu M, Muntean D, Dégi J, Boldea MV, et al. Comparative Evaluation of qnrA, qnrB, and qnrS Genes in Enterobacteriaceae Ciprofloxacin-Resistant Cases, in Swine Units and a Hospital from Western Romania. Antibiotics (Basel) 2020;9:698. doi: 10.3390/antibiotics9100698.
19. Shi W, Li K, Ji Y, Jiang Q, Wang Y, Shi M, et al. Carbapenem and cefoxitin resistance of Klebsiella pneumoniae strains associated with porin OmpK36 loss and DHA-1 β-lactamase production. Braz J Microbiol 2013;44:435-42. doi: 10.1590/S1517-83822013000200015.
20. El-Domany RA, Awadalla OA, Shabana SA, El-Dardir MA, Emara M. Analysis of the Correlation Between Antibiotic Resistance Patterns and Virulence Determinants in Pathogenic Klebsiella pneumoniae Isolates from Egypt. Microb Drug Resist 2021;27:727-39. doi: 10.1089/mdr.2020.0236.
21. Wang J, Yan Y, Xue X, Wang K, Shen D. Comparison of pyogenic liver abscesses caused by hypermucoviscous Klebsiella pneumoniae and non-Klebsiella pneumoniae pathogens in Beijing: a retrospective analysis. J Int Med Res 2013;41:1088-97. doi: 10.1177/0300060513487645.
22. Abdallah KS, Cao Y, Wei DJ. Epidemiologic Investigation of Extra-intestinal pathogenic E. coli (ExPEC) based on PCR phylogenetic group and fimH single nucleotide polymorphisms (SNPs) in China. Int J Mol Epidemiol Genet 2011;2:339-53.
23. Candan ED, Aksöz N. Klebsiella pneumoniae: characteristics of carbapenem resistance and virulence factors. Acta Biochim Pol 2015;62:867-74. doi: 10.18388/abp.2015_1148.
24. Zheng JX, Lin ZW, Sun X, Lin WH, Chen Z, Wu Y, et al. Overexpression of OqxAB and MacAB efflux pumps contributes to eravacycline resistance and heteroresistance in clinical isolates of Klebsiella pneumoniae. Emerg Microbes Infect 2018;7:139. doi: 10.1038/s41426-018-0141-y.
25. Compain F, Babosan A, Brisse S, Genel N, Audo J, Ailloud F, et al. Multiplex PCR for detection of seven virulence factors and K1/K2 capsular serotypes of Klebsiella pneumoniae. J Clin Microbiol 2014;52:4377-80. doi: 10.1128/JCM.02316-14.
26. Biran D, Sura T, Otto A, Yair Y, Becher D, Ron EZ. Surviving Serum: the Escherichia coli iss Gene of Extraintestinal Pathogenic E. coli Is Required for the Synthesis of Group 4 Capsule. Infect Immun 2021;89:e0031621. doi: 10.1128/IAI.00316-21.
27. Zurfluh K, Nüesch-Inderbinen M, Morach M, Zihler Berner A, Hächler H, Stephan R. Extended-spectrum-β-lactamase-producing Enterobacteriaceae isolated from vegetables imported from the Dominican Republic, India, Thailand, and Vietnam. Appl Environ Microbiol 2015;81:3115-20. doi: 10.1128/AEM.00258-15.
28. Han R, Shi Q, Wu S, Yin D, Peng M, Dong D, et al. Dissemination of Carbapenemases (KPC, NDM, OXA-48, IMP, and VIM) Among Carbapenem-Resistant Enterobacteriaceae Isolated From Adult and Children Patients in China. Front Cell Infect Microbiol 2020;10:e314. doi: 10.3389/fcimb.2020.00314.
29. Bahmani N. Detection of VIM-1, VIM-2 and IMP-1 metallo- β-lactamase genes in Klebsiella pneumoniae isolated from clinical samples in Sanandaj, Kurdistan, west of Iran. Iran J Microbiol 2019;11:225-31.
30. Salah FD, Soubeiga ST, Ouattara AK, Sadji AY, Metuor-Dabire A, Obiri-Yeboah D, et al. Distribution of quinolone resistance gene (qnr) in ESBL-producing Escherichia coli and Klebsiella spp. in Lomé, Togo. Antimicrob Resist Infect Control 2019;8:e104. doi: 10.1186/s13756-019-0552-0.
31. Malek Jamshidi MR, Zandi H, Eftekhar F. Correlation of quinolone-

resistance, qnr genes and integron carriage in multidrug-resistant community isolates of Klebsiella spp. Iran J Basic Med Sci 2019;22:1387-91. doi: 10.22038/IJBMS.2019.13985.

32. Latifi B, Tajbakhsh S, Ahadi L, Yousefi F. Coexistence of aminoglycoside resistance genes in CTX-M-producing isolates of Klebsiella pneumoniae in Bushehr province, Iran. Iran J Microbiol 2021;13:161-70. doi: 10.18502/ijm.v13i2.5975.
33. Kuş H, Arslan U, Türk Dağı H, Fındık D. Investigation of various virulence factors of Klebsiella pneumoniae strains isolated from nosocomial infections. Mikrobiyol Bul 2017;51:329-39. Turkish. doi: 10.5578/mb.59716.
34. Gharrah MM, Mostafa El-Mahdy A, Barwa RF. Association between Virulence Factors and Extended Spectrum Beta-Lactamase Producing Klebsiella pneumoniae Compared to Nonproducing Isolates. Interdiscip Perspect Infect Dis 2017;2017:7279830. doi: 10.1155/2017/7279830.
35. Davies YM, Cunha MP, Oliveira MG, Oliveira MC, Philadelpho N, Romero DC, et al. Virulence and antimicrobial resistance of Klebsiella pneumoniae isolated from passerine and psittacine birds. Avian Pathol 2016;45:194-201. doi: 10.1080/03079457.2016.1142066.
36. Candan ED, Aksöz N. Klebsiella pneumoniae: characteristics of carbapenem resistance and virulence factors. Acta Biochim Pol 2015;62:867-74. doi: 10.18388/abp.2015_1148.
37. Remya PA, Shanthi M, Sekar U. Characterisation of virulence genes associated with pathogenicity in Klebsiella pneumoniae. Indian J Med Microbiol 2019;37:210-8. doi: 10.4103/ijmm.IJMM_19_157.