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ORIGINAL ARTICLE

Emergence of co-existence of *bla*$_{NDM}$ with *rmtC* and *qnrB* genes in clinical carbapenem-resistant *Klebsiella pneumoniae* isolates in burning center from southeast of Iran

Somayeh Kiaei [1,2] · Mohammad Moradi [1] · Hossein Hosseini Nave [1] · Zahra Hashemizadeh [2] · Majid Taati-Moghadam [2] · Davood Kalantar-Neyestanaki [1]

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Abstract

Dissemination of carbapenemase-producing *Klebsiella pneumoniae* along with 16S rRNA methyltransferase (16S-RMTase) has been caused as a great concern for healthcare settings. The aim of this study was to investigate the prevalence of resistance genes among *K. pneumoniae* isolates. During October 2015 to February 2016, 30 non-duplicative *K. pneumoniae* strains were isolated from clinical specimens in a burn center in Kerman, Iran. Antibiotic susceptibility tests of isolates, carbapenemase, extended-spectrum beta-lactamases (ESBLs) and AmpC beta-lactamase-producing isolates were determined by phenotypic methods. The beta-lactamase, *oqxA/B* efflux pumps, *qnr A*, *B*, *S*, 16S-RMTase (*rmt A*, *B*, and C), and *mcr-1* resistance genes were determined by PCR. Enterobacterial repetitive intergenic consensus (ERIC)-PCR was used for molecular typing. According to our findings, tigecycline has been shown the most active agent against *K. pneumoniae* isolates. Antibiotic resistance genes, *bla*$_{TEM-1}$, *bla*$_{SHV-12}$, *bla*$_{CTX-M-15}$, *bla*$_{CTX-M-2}$, *bla*$_{NDM-1}$, *bla*$_{FOX}$, *bla*$_{MOX}$, *bla*$_{EBC}$, *bla*$_{ACC}$, *bla*$_{CIT}$, *rmtC*, *qnrB*, *qnrS*, *oqxA*, and *oqxB*, were detected in 11 (36.7%), 13 (43.3%), 11 (36.6%), 5 (16.6%), 9 (30%), 1 (3.3%), 1 (3.3%), 1 (3.3%), 1 (3.3%), 2 (6.7%), 1 (3.3%), 9 (30%), 2 (6.7%), 18 (60%), and 13 (43.3%) of isolates, respectively. The *bla*$_{NDM-1}$ with *rmtC* was simultaneously observed in one isolate. ERIC-PCR results revealed 25 distinct patterns in eight clusters (A–H) and five singletons. This study highlights the high prevalence of *bla*$_{NDM}$ and emergence of *rmtC* among carbapenem-resistant *K. pneumoniae*. The resistance genes are often co-located on the conjugative plasmids, so it might be the reason of the rapid spread of them. The prevalence of multidrug-resistant *K. pneumoniae* isolates limits the available treatment options and presents tremendous challenges to public health.

Introduction

Emergence of carbapenem-resistant *Enterobacteriaceae* (CRE), especially carbapenem-resistant *Klebsiella pneumoniae*, is a major public health concern worldwide (Munoz-Price et al. 2013). The most effective antibiotics against carbapenem-resistant *K. pneumoniae* isolates are colistin and tigecycline (Nordmann et al. 2009). Resistance to β-lactam antibiotics in *Enterobacteriaceae* has been increasingly observed with the production of carbapenemases, extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases, overexpression of efflux pumps, and loss of porins (HU et al. 2014). CRE, particular New Delhi metallo-beta-lactamase (NDM)-producing *K. pneumoniae*, is one of the most potential gram-negative pathogens in hospital settings (Munoz-Price et al. 2013; Cantón et al. 2012). *K. pneumoniae* harbors putative efflux pump genes such as *oqxA/B*, which are originally located on conjugative plasmids, conferring resistance to antibacterial agents, including quinolones and chloramphenicol (Ruiz et al. 2012; Hansen et al. 2007). Previous studies have shown that mutations in DNA gyrase and DNA topoisomerase IV have caused fluoroquinolone resistance (Hansen et al. 2007). Although, plasmid-mediated quinolone resistance (PMQR), harboring *qnr* and *oqxAB* genes, has decreased fluoroquinolone susceptibility (Ruiz et al. 2012; Hansen et al. 2007). Furthermore, the *qnr* genes such as *qnrA*, *qnrS*, and *qnrB* encoding proteins protect topoisomerases from fluoroquinolone antibiotics (Ruiz et al. 2012). Almost all reported aminoglycoside resistance mechanisms are mediated by 16S rRNA methyltransferase (16S-RMTase) among carbapenemases and

✉ Davood Kalantar-Neyestanaki
D.kalantar@kmu.ac.ir

[1] Department of Microbiology and Virology, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran

[2] Student Research Committee, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran

extended-spectrum β-lactamase (ESBL)-producing gram-negative bacteria (Bueno et al. 2013).

Colistin is an important antibiotic agent in treating infections caused by carbapenem-resistant bacteria. Recently, colistin-resistant isolates have been reported worldwide by intrinsic resistance such as mutations in the two-component regulatory systems PmrAB, PhoPQ and plasmid-mediated transferable colistin resistance gene, *mcr-1* (Liu et al. 2016; Quan et al. 2017).

Molecular typing methods are usually used for control and monitor of nosocomial infections in healthcare settings (Messai et al. 2008). One of the most traditional molecular typing systems, providing an overview of microbial source tracking methods, is the enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) (Ben-Hamouda et al. 2003). The method has been introduced as a short consuming and rapid method to predict genetic diversity among multidrug resistance (MDR) isolates in the hospital settings (Wasfi et al. 2016). The aim of this study was to determine the antimicrobial resistance profiles, mechanisms of resistance, and genetic relationship among 30 *K. pneumoniae* isolates which were collected from hospitalized patients in a burn center in Kerman, Iran.

Material and methods

Bacteria isolates

During this study (October 2015 to February 2016), 30 non-duplicative isolates of *K. pneumoniae* were recovered from different clinical specimens from hospitalized patients in burn center (Shafa-Hospital), Kerman, Iran. The category of hospitalized patients was in accordance with the current Centers for Disease Control and Prevention (CDC) recommendations (Horan et al. 2008). All of the isolates were identified as *K. pneumoniae* by microbiological and biochemical standard tests including gram-negative staining, catalase positive, oxidase negative, indole negative, methyl red negative, Voges-Proskauer positive, citrate utilization positive, and urease positive (Mahon et al. 2007).

Antibiotic susceptibility testing

Antibacterial susceptibility test of isolates to different antibiotic disks (ROSCO, Co, Denmark) including cefepime (30 μg), cefotaxime (30 μg), cefoxitin (30 μg), ceftazidime (30 μg), ceftizoxime (30 μg), cefpodoxime (10 μg), imipenem (10 μg), meropenem (10 μg), ertapenem (10 μg), gentamicin (10 μg), amikacin (30 μg), ciprofloxacin (5 μg), norfloxacin (10 μg), and fosfomycin (200 μg) was determined by disk diffusion method on Müller-Hinton agar media (CONDA, Co, Spain) according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (http://www.eucast.org/clinical_breakpoints/). Minimum inhibitory concentration (MIC) of isolates to cefotaxime, cefepime, imipenem, and colistin was determined by microbroth dilution method according to the Clinical & Laboratory Standards Institute (CLSI 2017). The MIC of isolates to tigecycline was also determined by broth microdilution according to the EUCAST standard breakpoints recommendations. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as standard strains in antibacterial susceptibility testing.

Detection of ESBL-, AmpC-, and carbapenemase-producing isolates

ESBL-producing isolates were determined by double disk method by four disks including ceftazidime (30 μg), cefotaxime (30 μg), cefpodoxime (10 μg), and amoxicillin/clavulanic acid (20 μg/10 μg) according to the British Society for Antimicrobial Chemotherapy (BSAC 2018) (http://www.bsac.org.uk) recommendation and EUCAST.

AmpC-β-lactamase and carbapenemase-producing isolates were determined by AmpC disk test and modified Hodge test (MHT) as described previously, respectively (Black et al. 2005; CLSI 2017). *K. pneumoniae* ATCC 700603 was used as a positive ESBL control. *Enterobacter* spp. KEJ-1 with *bla*$_{NDM}$ (GenBank accession no. KP347135) and *Enterobacter* spp. KEJ-3 with *bla*$_{ACT}$ (GenBank accession no. KP347137) were used as positive control in MHT and AmpC disk methods, respectively. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as negative control in confirmatory tests for detection of ESBL-, AmpC-, and carbapenemase-producing isolates.

The genomic DNA extraction

The genomic DNA extraction using Exgene Clinic SV (GeneALL, Co, Seoul, Korea, Kat: 106-152) was according to manufacturer's guidelines.

Detection of *bla*, *oqxA/B* efflux pumps, *mcr-1*, *qnr*, **and 16S RNA methylase genes was by PCR**.

The antibiotic resistance genes including *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX-M}$, *bla*$_{KPC}$, *bla*$_{OXA-48}$, *bla*$_{IMP}$, *bla*$_{VIM}$, *bla*$_{NDM}$, *oqxA/B* efflux pumps, *mcr-1*, *qnrA*, *qnrB*, *qnrS*, *rmtA*, *rmtB*, and *rmtC* were detected by PCR technique. The primers of antibiotic resistance genes that were used in this study are listed in Table 1. PCR amplification was performed in a total volume of 25 μL containing 0.5 μL of each primer (10 pmol/L concentration), 12.5 μL of Taq DNA Polymerase Master Mix RED (AMPLIQON, Inc., Denmark), 1 μL of DNA, and

Table 1 Oligonucleotide primers used in this study

Target genes	Primer sequence (5′-3′)	Annealing temperature (°C)	Product size (bp)	Reference
bla_{CTX-M}	F-ATGTGCAGYACCAGTAARGTKATGGC R-TGGGTRAARTARGTSACCAGAAYCAGCGG	58	593	(Monstein et al. 2007)
bla_{TEM}	F-ATAAAATTCTTGAAGACGAAA R-GACAGTTACCAATGCTTAATC	45	1080	(Yun-Tae et al. 2006)
bla_{SHV}	F-TCGTTATGCGTTATATTCGCC R-GGTTAGCGTTGCCAGTGCT	58	866	(Yun-Tae et al. 2006)
bla_{KPC}	F-CGTCTAGTTCTGCTGTCTTG R-CTTGTCATCCTTGTTAGGCG	58	798	(Daoud et al. 2015)
bla_{OXA-48}	F-GCGTGGTTAAGGATGAACAC R-CATCAAGTTCAACCCAACCG	58	438	(Poirel et al. 2011)
bla_{VIM}	F-GATGGTGTTTGGTCGCATA R-CGAATGCGCAGCACCAG	53	390	(Daoud et al. 2015)
bla_{IMP}	F-GGAATAGAGTGGCTTAAYTCTC R-GGTTTAAYAAAACAACCACC	58	232	(Daoud et al. 2015)
bla_{NDM}	F-GAATTCGCCCCATATTTTTGC R-AACGCCTCTGTCACATCGAAAT	59	977	(Qin et al. 2016)
oqxA	F-CTCGGCGCGATGATGCT R-CCACTCTTCACGGGAGACGA	57	392	(Kim et al. 2009)
oqxB	F-TTCTCCCCCGGCGGGAAGTAC F-CTCGGCCATTTTGGCGCGTA	56	512	(Kim et al. 2009)
rmtA	F-CTAGCGTCCATCCTTTCCTC R-TTTGCTTCCATGCCCTTGCC	56	635	(Fritsche et al. 2008)
rmtB	F-CCCAAACAGACCGTAGAGGC R-CTCAAACTCGGCGGGCAAGC	56	585	(Guo et al. 2016)
rmtC	F-CGAAGAAGTAACAGCCAAAG R-AATCCCAACATCTCTCCCACT	61	711	(Hidalgo et al. 2018)
qnrA	F-ATTTCTCACGCCAGGATTTG R-GATCGGCAAAGGTTAGGTCA	60	516	(Robicsek et al. 2012)
qnrB	F-GATCGTGAAAGCCAGAAAGG R-ACGATGCCTGGTAGTTGTCC	60	469	(Robicsek et al. 2012)
qnrS	F-ACGACATTCGTCAACTGCAA R-TAAATTGGCACCCTGTAGGC	60	417	(Robicsek et al. 2012)
mcr-1	F-CGGTCAGTCCGTTTGTTC R-CTTGGTCGGTCTGTAGGG	53	309	(Liu et al. 2016)

10.5 μL of DNase and RNase-free water (SinaClon, BioScience, Co, Iran) in a FlexCycler PCR Thermal Cycler (Analytik Jena, Germany) under the following conditions: initial denaturation at 95 °C for 3 min followed by 30 cycles of denaturation at 95 °C for 1 min, annealing at 51–61 °C for 45 s (Table 1), extension at 72 °C for 1 min, and the final extension step was continued for 5 min at 72 °C. The PCR products were electrophoresed on 1.5% agarose gel containing DNA Green Viewer™ dye (Pars tous Biotechnology, Co, Iran) in 0.5× Tris-EDTA-boric acid buffer (TBE). PCR products from bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{NDM}, and *rmtC* were sequenced in both directions (Macrogen, South Korea). The nucleotide sequences were compared with the GenBank database for homologous nucleotide sequences by BLAST program (www.ncbi.nih.gov/BLAST program). The AmpC beta-lactamase genes including bla_{FOX}, bla_{MOX}, bla_{EBC}, bla_{ACC}, bla_{DHA}, and bla_{CIT} were detected using multiplex PCR as previously described (Pérez-Pérez and Hanson 2002) and *mcr-1* was detected among isolates with reduced susceptibility to colistin by PCR.

Molecular typing of isolates by ERIC-PCR

The ERIC-PCR was used for molecular typing of isolates using ERIC2 primer 5′-AAGTAAGTGACTGGGGTGAGC-3′ (Meacham et al. 2003). The ERIC-PCR amplification was carried out in FlexCycler PCR Thermal Cycler (Analytik Jena, Germany) using Taq DNA Polymerase Master Mix RED (AMPLIQON, Inc., Denmark). PCR amplification was performed in a total volume of 25 μL containing 1 μL of primer (10 pmol/L concentration), 12.5 μL of Taq DNA Polymerase Master Mix RED (AMPLIQON, Inc., Denmark), 1 μL of DNA, and 10.5 μL of DNase and RNase-free water (SinaClon, BioScience, Co, Iran) in a FlexCycler PCR Thermal Cycler (Analytik Jena, Germany) under the following conditions: initial denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 2 min, and the final extension step was continued for 10 min at 72 °C. The PCR products were electrophoresed on 1.5% agarose gel containing DNA Green Viewer dye in 0.5× TBE buffer and the results

were analyzed in http://insilico.ehu.eus/dice_upgma/. Cut off of 70% was used to discriminate among the isolates.

Results

Clinical samples

Clinical samples were recovered from specimens of burning wounds 4 (13.3%), urine specimens 17 (56.7%), blood 3 (10%), and bronchoalveolar lavage (BAL) 6 (20%).

Antibiotic susceptibility testing

In this study, the rate of resistance to antibiotics was as follows: 15 (50%) of isolates were resistant to cefotaxime and ceftazidime, 16 (53.3%) to cefpodoxime and ceftizoxime, 14 (46.7%) to cefepime, 13 (43.3%) to cefoxitine, 8 (26.7%) to norfloxacin and ciprofloxacin, 14 (46.7%) to amikacin, 10 (33.3%) to gentamicin, 8 (26.7%) to fosfomycin, 8 (26.7%) to meropenem, 7 (23.3%) to imipenem, and 9 (30%) to ertapenem. In this study, the decrease of susceptibility to colistin was observed in 7 (23.3%) of isolates, as follows: one isolate with MIC = 16 μg/mL, four isolates with MIC = 4 μg/mL, and two isolates with MIC = 2 μg/mL, although, the 23 (76.6%) of isolates were shown MIC ≤ 0.5 μg/mL. Our findings showed that all isolates were susceptible to tigecycline with MIC ≤ 1 μg/mL. Antibiotic resistance patterns, MIC to imipenem, cefotaxime, cefepime, colistin, and genetic characterizations of 19 multidrug-resistant isolates have been shown in Table 2.

Phenotypic confirmatory tests

In this study, 19 (63.3%), 5 (16.7%), and 5 (16.7%) of isolates were considered as ESBL-, AmpC-, and carbapenemase-producing by phenotypic methods, respectively. In our findings, among the isolates were resistant to amoxicillin/clavulanate, cefotaxime, ceftazidime, and cefpodoxime the inhibition zone was not observed in 5 (16.7%) isolates in MDDST; these 5 isolates (strains 2, 3, 9, 17, 29, Table 2) showed positive results for AmpC ($n = 3$) and MHT ($n = 5$).

Molecular characterization of resistance genes

The *ESBLs* genes including bla_{TEM}, $bla_{\mathrm{CTX\text{-}M}}$, and bla_{SHV} were detected in 11 (57.8%), 16 (84.2%), and 13 (68.4%) of ESBL-producing isolates ($n = 19$), respectively. bla_{NDM} was detected in the all carbapenem-resistant isolates ($n = 9$). One of the NDM-producing *K. pneumoniae* isolates harbored *rmtC* gene. Sequencing of the full length *bla* genes PCR products confirmed that the bla_{NDM}, bla_{TEM}, $bla_{\mathrm{CTX\text{-}M}}$, and bla_{SHV} genes were 100% identical to $bla_{\mathrm{NDM\text{-}1}}$, $bla_{\mathrm{TEM\text{-}1}}$, $bla_{\mathrm{CTX\text{-}M\text{-}15}}$ ($n = 11$), $bla_{\mathrm{CTX\text{-}M\text{-}2}}$($n = 5$), and $bla_{\mathrm{SHV\text{-}12}}$ reported in GenBank. The bla_{NDM}, bla_{TEM}, $bla_{\mathrm{CTX\text{-}M}}$, bla_{SHV}, and *rmtC* sequences were submitted to the GenBank by accession numbers KY856828, MG515601, MG515591, MG515598, and KY849821, respectively. The *oqxA*, *oqxB*, *qnrB*, and *qnrS* genes were detected in 18 (60%), 13 (43.3%), 9 (30%), and 2 (6.7%) of isolates, respectively. AmpC β-lactamase genes including bla_{FOX}, bla_{MOX}, bla_{EBC}, and bla_{ACC} were detected in 1 (3.3%) of isolates, respectively, and bla_{CIT} was detected in 2 (6.7%) of isolates. The genetic characterization of ESBL-, AmpC-, and NDM-producing *K. pneumoniae* is summarized in Table 2. All isolates were negative for bla_{VIM}, bla_{IMP}, bla_{KPC}, $bla_{\mathrm{OXA\text{-}48}}$, bla_{DHA}, *rmtA*, *rmtB*, *qnrA*, and *mcr-1* genes.

Molecular typing

The 30 *K. pneumoniae* isolates were divided into eight clusters (A–H) by ERIC-PCR methods. Five isolates from clusters A and B; two isolates from clusters C, F, G, and H; three isolates from clusters D and E; and five isolates were considered as singleton and one isolate was non-typeable. ERIC-PCR among 30 *K. pneumoniae* isolates revealed different distinct patterns (Figs. 1 and 2).

Discussion

The continued emergence and dissemination of carbapenemase-producing *Enterobacteriaceae* is a serious problem among clinicians and public health systems from different parts of the world (Shibl et al. 2013). The important reservoir for carbapenemase-producing isolates might be India and Pakistan, although it has been suggested that the Middle East region is considered as a secondary reservoir for the spread of these bacteria (Shibl et al. 2012; Nordmann et al. 2011). Similar to our findings, Nobari et al. reported a study in which the resistance rates to cephalosporins were as follows: 75, 76.6, and 78.8% isolates were resistant to cefepime, ceftazidime, and cefotaxime, respectively. They showed that imipenem has the highest activity against *K. pneumoniae* isolates and the percent of resistance to other antimicrobial agents was as follows: ciprofloxacin, 73.3% and amikacin, 22.2% (Nobari et al. 2014). In the current study, colistin and tigecycline have shown the most antibacterial activity against *K. pneumoniae* isolates. Similar to our findings, Samonis et al. conducted among 65 MDR *K. pneumoniae* isolates, susceptibility to tigecycline, colistin, carbapenems were 84.6, 75.4, and 21.5%, respectively (Samonis et al. 2012). In our study, despite reduced susceptibility to colistin, *mcr-1* gene was not detected. However, resistance to colistin in gram-negative bacteria might be associated to intrinsic resistance such as mutations in the two-component regulatory systems PmrAB

Table 2 Antimicrobial susceptibility testing and distribution of resistance genes among 19 MDR clinical isolates of *K. pneumoniae*

Strains/ ERIC	Hospital settings	Specimen source	Antimicrobial resistance	MIC (μg/mL)				Resistance genes	ESBLs	MHT	AmpC
				IMI	CTX	FEP	CO				
Strain 1/S	Internal	Urine	AMI, FO, CO[a]	≤ 1	≤ 1	≤ 2	2	*oqxA*	–	–	–
Strain 2/D	Burning	Wound	CTX, CAZ, GEN, AMI, IMI, CIP, MRP, CPD, CFO, ZOX, FEP, NORFX	64	1024	64	≤ 0.5	bla_{NDM}, $bla_{CTX\text{-}M}$, *oqxA*,*oqxB*, *qnrB*, *qnrS*	–	+	–
Strain 3/H	Burning	Wound	CTX, CAZ, AMI, IMI, CIP, MRP, CPD, CFO, ZOX, FEP	16	2048	1024	≤ 0.5	bla_{NDM}, $bla_{CTX\text{-}M}$, bla_{OXA}, bla_{SHV}, bla_{TEM}, *oqxA*, *oqxB*,	–	+	+
Strain 4/H	ICU	BAL	CTX, CAZ, GEN, AMI, IMI, FO, MRP, CPD, CO[a], CFO, ZOX, FEP, NORFX	8	512	256	16	bla_{NDM}, $bla_{CTX\text{-}M}$, bla_{TEM}, *oqxA*, *oqxB*	+	–	–
Strain 5/D	ICU	Urine	CTX, CAZ, AMI, CPD, CFO, ZOX, FEP	4	2048	256	≤ 0.5	bla_{NDM}, $bla_{CTX\text{-}M}$, bla_{SHV}, bla_{TEM}	+	–	–
Strain 6/B	Internal	Urine	CAZ, GEN, AMI, MRP, CPD, ZOX	≤ 1	≤ 1	≤ 2	≤ 0.5	$bla_{CTX\text{-}M}$, bla_{TEM}, *oqxA*, *oqxB*	+	–	–
Strain 7/B	Internal	Urine	CTX, CAZ, GEN, AMI, IMI, CIP, FO, MRP, CPD, CO[a], CFO, ZOX, FEP, NORFX	16	1024	64	4	bla_{NDM}, $bla_{CTX\text{-}M}$, bla_{TEM}, *oqxA*, *oqxB*, *qnrS*	+	–	–
Strain 8/N	ICU	BAL	AMI, FO, CO[a]	≤ 1	≤ 1	≤ 2	2	*oqxA*, *oqxB*, *qnrB*	–	–	–
Strain 9/B	Burning	Wound	CTX, CAZ, GEN, AMI, IMI, FO, MRP, CPD, CO[a], CFO, ZOX, FEP	32	2048	1024	4	bla_{NDM}, $bla_{CTX\text{-}M}$, bla_{SHV} bla_{TEM}, *oqxA*, *oqxB*	–	+	–
Strain 11/C	Surgery	Blood	CTX, CAZ, GEN, CIP, CPD, ZOX, FEP, NORFX	≤ 1	1024	64	≤ 0.5	$bla_{CTX\text{-}M}$, bla_{SHV}, bla_{TEM}, *oqxA*, *qnrB*	+	–	–
Strain 14/B	Internal	Urine	CTX, CAZ, GEN, AMI, CIP, FO, CPD, CO[a], CFO, ZOX, FEP, NORFX	≤ 1	2048	2048	4	$bla_{CTX\text{-}M}$, bla_{SHV}, bla_{TEM}, *oqxA*, *oqxB*, *qnrB*	+	–	+
Strain 15/S	Internal	Urine	CTX, CAZ, CPD, ZOX, FEP	≤ 1	2048	1024	≤ 0.5	$bla_{CTX\text{-}M}$, bla_{SHV}, bla_{TEM}, *oqxA*, *qnrB*	+	–	–
Strain 17/E	Internal	Urine	CTX, CAZ, GEN, AMI, IMI, MRP, CPD, CFO, ZOX, FEP	8	128	32	≤ 0.5	bla_{NDM}, $bla_{CTX\text{-}M}$, bla_{SHV}, *oqxA*, *oqxB*, *rmtC*, *qnrB*	–	+	+
Strain 21/A	ICU	Urine	CTX, CAZ, CPD, CFO, ZOX	≤ 1	64	≤ 2	≤ 0.5	$bla_{CTX\text{-}M}$, bla_{SHV}, *oqxA*, *qnrB*	+	–	–
Strain 22/S	Internal	Urine	CTX, CFO	≤ 1	64	≤ 2	≤ 0.5	–	–	–	+
Strain 24/E	ICU	Blood	CTX, CAZ, GEN, AMI, CIP, TIG, CPD, CFO, ZOX, FEP NORFX	4	256	512	≤ 0.5	bla_{NDM}, $bla_{CTX\text{-}M}$, bla_{SHV}, bla_{TEM}, *oqxA*, *oqxB*, *qnrB*	+	–	–
Strain27/G	Internal	Urine	AMI, FO, CPD, CO[a], CFO, ZOX, FEP	≤ 1	≤ 1	512	4	$bla_{CTX\text{-}M}$, bla_{SHV}, bla_{TEM}, *oqxA*, *oqxB*	+	–	–
Strain 29/F	Surgery	Urine	CTX, CAZ, AMI, IMI, CIP,MRP, CPD, CFO, ZOX, FEP, NORFX	64	2048	512	≤ 0.5	bla_{NDM}, $bla_{CTX\text{-}M}$, bla_{SHV}, *oqxA*, *oqxB*	–	+	+
Strain 30/F	Internal	Urine	CTX, CAZ, GEN, CIP, CPD, FEP, ZOX, NORFX	≤ 1	1024	512	≤ 0.5	$bla_{CTX\text{-}M}$, bla_{SHV}, *oqxA*, *qnrB*	+	–	–

IMI imipenem, *MRP* meropenem, *ERP* ertapenem, *CPD* cefpodoxime, *CTX* cefotaxime, *CAZ* ceftazidime, *ZOX* ceftizoxime, *CFO* cefoxitine, *FEP* cefepime, *CIP* ciprofloxacine, *NORFX* norfloxacin, *GEN* gentamicin, *AMI* amikacin, CO colistin, *TIG* tigecycline, *FO* fosfomycin, *Strains/ERIC* strain/cluster in ERIC-PCR. MIC to other isolates ($n = 11$): IMI ≤ 0.5 μg/mL, CTX and FEP ≤ 1 μg/mL.

[a] The colistin susceptibility was reduced in these isolates

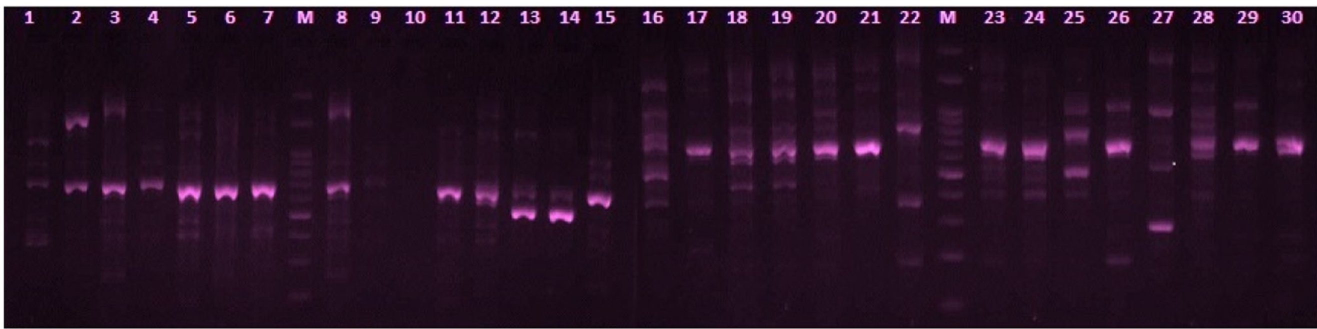

Fig. 1 Agarose gel electrophoresis of ERIC-PCR products for 30 clinical isolates of *Klebsiella pneumoniae*

and PhoPQ (Quan et al. 2017). One of the most prevalent mechanisms for resistance to colistin is modifications of bacterial lipopolysaccharide, which result in the reduction of colistin affinity (Liu et al. 2016; Quan et al. 2017). The present findings indicated that among cefoxitin-resistant isolates, only 5 (16.7%) of isolates were AmpC positive by AmpC disk test method. Helmy et al. observed the AmpC disk test positive among one out of three cefoxitin-resistant *K. pneumoniae* (Helmy and Wasfi 2014); therefore, cefoxitin resistance in non-AmpC-producing *K. pneumoniae* strains in our study may be caused by the development of deficient mutants in porin-encoding genes (Lee et al. 2007). Nevertheless, Hernandez-Alles et al. demonstrated that insertion sequence interruption of porin-encoding genes might also contribute to resistance to cefoxitin (Hernandez-Alles 1999). In the current study, among ESBL-producing isolates, bla_{CTX-M} 16(84.2%) was more prevalent than the other *bla* genes, which was in agreement with previous findings. In a study in Korea, bla_{CTX-M} (80.6%) was the more prevalent *bla* gene among ESBL-producing *K. pneumoniae* (Cha et al. 2018), although Feizabadi et al. and Cheddie et al. showed bla_{SHV} was the more prevalent *bla* gene among *K. pneumoniae* isolates (Feizabadi et al. 2010; Cheddie et al. 2017). In other countries, bla_{VIM} and bla_{IMP} carbapenemase-producing *Enterobacteriaceae* have

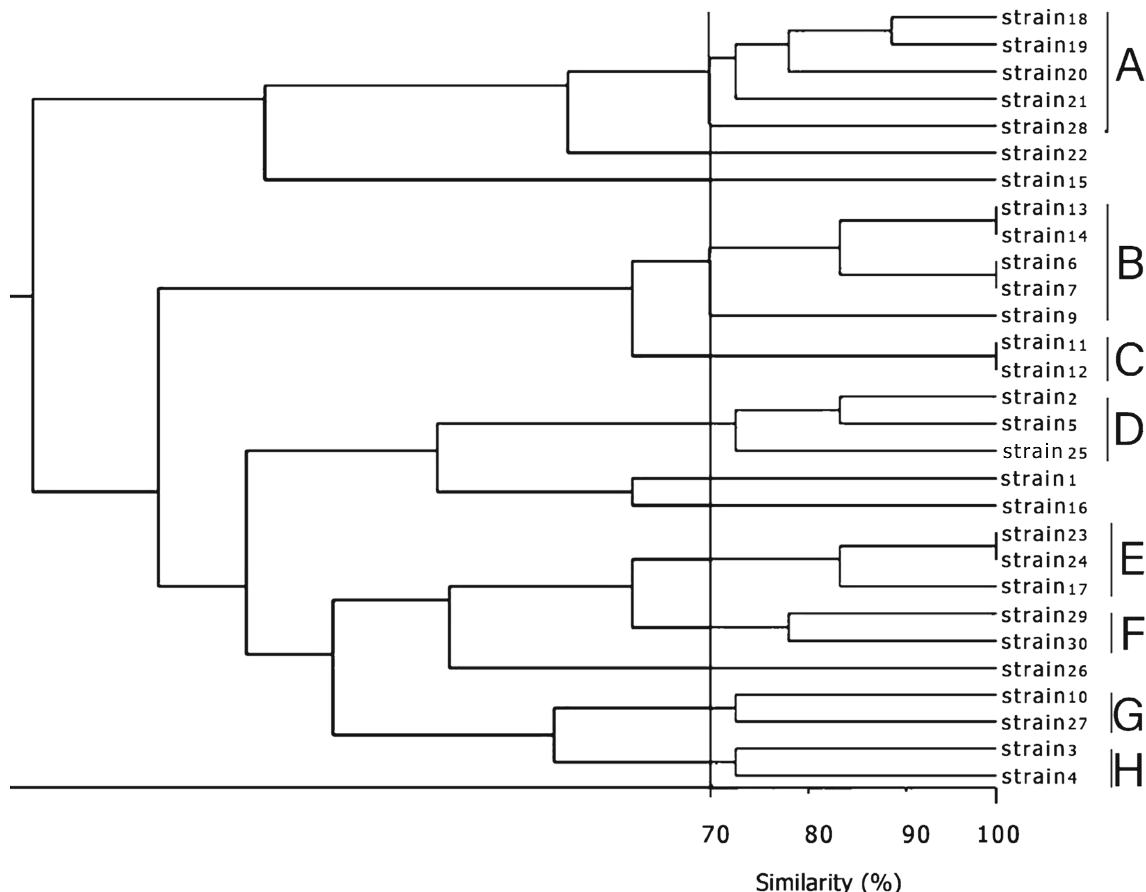

Fig. 2 Corresponding dendrogram generated with UPGMA clustering method for 30 clinical isolates of *Klebsiella pneumoniae*

been reported, although these carbapenemase genes were not reported in this study(Azimi et al. 2014). The most important finding in the present study was high prevalence of bla_{NDM-1} among carbapenem-resistant isolates.

Within a few years, the rapid global spread of 16S-RMTase producers has been associated with widespread dissemination among members of *Enterobacteriaceae* that produce NDM-metallo-β-lactamase (Samonis et al. 2012; Helmy and Wasfi 2014), since these resistant determinants are usually harbored on the same conjugative plasmid among *Enterobacteriaceae* (Lee et al. 2007; Hernández-Allés et al. 1999). In this study, among aminoglycoside resistance genes in clinical isolates, one isolate carried *rmtC* gene. 16S-RMTase genes, in particular, *rmtC*, have been so far accumulated on conjugative plasmids, integrons, and other genetic elements that are mainly related to rapid dissemination of β-lactamase genes (Wachino et al. 2006; Toleman and Walsh 2011). At this study, we showed the presence of *rmtC* and the β-lactamase genes including bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{SHV-12}, *qnrB*, *oqxA*, and *oqxB* in one isolate simultaneously. However, Jiang et al., Liu et al., and Poirel et al. showed that PMQR proteins including QnrA and QnrB were coproduced with 16S-RMTases (Jiang et al. 2010; Liu et al. 2008; Poirel et al. 2011). In this study, *qnr* genes were determined as follows: *qnrB* 9 (30%) and *qnrS* 2 (6.7%) of which all of them have one or two efflux pumps *oqxA/B* genes simultaneously. In this work, the prevalence of *qnrS* (6.7%) gene was significantly lower than that of the *qnrB* (30%) gene. Similar to our findings, Azadpour et al. reported the prevalence of *qnrS* (5.55%) was much lower than that of the *qnrB* (88.9%) (Azadpour et al. 2014). Molecular typing of clinical multidrug-resistant *K. pneumoniae* isolates is the powerful tool to characterize and prevent the spread of infections in healthcare systems (Wasfi et al. 2016). In the current study, ERIC-PCR revealed 25 distinct patterns, so according to the different antimicrobial susceptibility profiles, resistance gene patterns as well as a wide dissemination of different clones through participant wards in the hospital, confirmed a high diversity and genetic transmission among *K. pneumoniae* isolates.

Conclusion

In this study, we described one *K. pneumoniae* isolate co-harboring bla_{NDM-1} and *rmtC* genes, which was isolated from urinary tract infection. Epidemiological typing results suggested the rise of different clones of *K. pneumoniae*, harboring various types of resistance genes, contribute to a wide reservoir of resistance genes among our hospital settings. Therefore, more surveillance on operational infection control policies is essential to prevent the outbreak of these bacteria in healthcare settings.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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References

Azadpour M, Soleimani Y, Rezaie F, Nikanpour E, Mahmoudvand H, Jahanbakhsh S (2014) Prevalence of *qnr* genes and antibiotic susceptibility patterns among clinical isolates of *Klebsiella Pneumoniae* in west of Iran. J Bacteriol Parasitol 5:1000202

Azimi L, Nordmann P, Lari AR, Bonnin RA (2014) First report of OXA-48-producing *Klebsiella pneumoniae* strains in Iran. GMS Hyg Infect Control 9:Doc07

Ben-Hamouda T, Foulon T, Ben-Cheikh-Masmoudi A, Fendri C, Belhadj O, Ben-Mahrez K (2003) Molecular epidemiology of an outbreak of multiresistant *Klebsiella pneumoniae* in a Tunisian neonatal ward. J Med Microbiol 52:427–433

Black JA, Moland ES, Thomson KS (2005) AmpC disk test for detection of plasmid-mediated AmpC β -lactamases in *Enterobacteriaceae* lacking chromosomal AmpC β-lactamases. J Clin Microbiol 43:3110–3113

British Society of Antimicrobial Chemotherapy (BSAC) (2018) BSAC to actively support the EUCAST disc diffusion method for antimicrobial susceptibility testing in preference to the current BSAC disc diffusion method. http://www.bsac.org.uk

Bueno MF, Francisco GR, O'Hara JA, de Oliveira Garcia D, Doi Y (2013) Coproduction of 16S rRNA methyltransferase RmtD or RmtG with KPC-2 and CTX-M group extended-spectrum β lactamases in *Klebsiella pneumoniae*. Antimicrob Agents Chemother 57:2397–2400

Cantón R, Akóva M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Miriagou V, Naas T, Rossolini GM, Samuelsen Ø (2012) Rapid evolution and spread of carbapenemases among *Enterobacteriaceae* in Europe. Clin Microbiol Infect 18:413–431

Cha MK, Kang CI, Kim SH, Chung DR, Peck KR, Lee NY, Song JH (2018) High prevalence of CTX-M-15-type extended-spectrum β-lactamase among AmpC β-lactamase-producing *Klebsiella pneumoniae* isolates causing bacteremia in Korea. Microb Drug Resist. https://doi.org/10.1089/mdr.2017.0362

Cheddie P, Dziva F, Akpaka PE (2017) Detection of a CTX-M group 2 beta-lactamase gene in a *Klebsiella pneumoniae* isolate from a tertiary care hospital, Trinidad and Tobago. Ann Clin Microbiol Antimicrob 16:33

CLSI (2017) Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute

Daoud Z, Salem Sokhn E, Masri K, Cheaito K, Haidar-Ahmad N, Matar GM, Doron S (2015) *Escherichia coli* isolated from urinary tract infections of Lebanese patients between 2005 and 2012: epidemiology and profiles of resistance. Front Med 2:26

European Committee on Antimicrobial Susceptibility Testing (2018) Breakpoints tables for interpretation of MICs and zones diametersVersion 8.0. http://www.eucast.org/clinical_breakpoints

Feizabadi MM, Delfani S, Raji N, Majnooni A, Aligholi M, Shahcheraghi F, Parvin M, Yadegarinia D (2010) Distribution of bla_{TEM}, bla_{SHV}, bla_{CTX-M} genes among clinical isolates of *Klebsiella pneumoniae* at Labbafinejad Hospital, Tehran, Iran. Microb Drug Resist 16:49–53

Fritsche TR, Castanheira M, Miller GH, Jones RN, Armstrong ES (2008) Detection of methyltransferases conferring high-level resistance to aminoglycosides in Enterobacteriaceae from Europe, North America, and Latin America. Antimicrob Agents Chemother 52: 1843–1845

Guo Y, Zhou H, Qin L, Pang Z, Qin T, Ren H, Pan Z, Zhou J (2016) Frequency, antimicrobial resistance and genetic diversity of *Klebsiella pneumoniae* in food samples. PLoS One 11:e0153561

Hansen LH, Jensen LB, Sørensen HI, Sørensen SJ (2007) Substrate specificity of the OqxAB multidrug resistance pump in *Escherichia coli* and selected enteric bacteria. J Antimicrob Chemother 60:145–147

Helmy MM, Wasfi R (2014) Phenotypic and molecular characterization of plasmid mediated AmpC β-lactamases among *Escherichia coli*, *Klebsiella* spp., and Proteus mirabilis isolated from urinary tract infections in Egyptian hospitals. Biomed Res Int 2014:171548

Hernández-Allés S, Benedí VJ, Martínez-Martínez L, Pascual Á, Aguilar A, Tomás JM, Albertí S (1999) Development of resistance during antimicrobial therapy caused by insertion sequence interruption of porin genes. Antimicrob Agents Chemother 43:937–939

Hidalgo L, Hopkins KL, Gutierrez B, Ovejero CM, Shukla S, Douthwaite S, Prasad KN, Woodford N, Gonzalez-Zorn B (2018) Association of the novel aminoglycoside resistance determinant RmtF with NDM carbapenemase in *Enterobacteriaceae* isolated in India and the UK. J Antimicrob Chemother 68:1543–1550

Horan TC, Andrus M, Dudeck MA (2008) CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control 36:309–332

Hu L, Zhong Q, Shang Y, Wang H, Ning C, Li Y, Hang Y, Xiong J, Wang X, Xu Y, Qin Z (2014) The prevalence of carbapenemase genes and plasmid-mediated quinolone resistance determinants in carbapenem-resistant *Enterobacteriaceae* from five teaching hospitals in Central China. Epidemiol Infect 142:1972–1977

Jiang Y, Yu D, Wei Z, Shen P, Zhou Z, Yu Y (2010) Complete nucleotide sequence of *Klebsiella pneumoniae* multidrug resistance plasmid pKP048, carrying bla_{KPC-2}, bla_{DHA-1}, *qnrB4*, and *armA*. Antimicrob Agents Chemother 54(9):3967

Kim HB, Wang M, Park CH, Kim EC, Jacoby GA, Hooper DC (2009) *oqxAB* encoding a multidrug efflux pump in human clinical isolates of *Enterobacteriaceae*. Antimicrob Agents Chemother 53:3582–3584

Lee CH, Chu C, Liu JW, Chen YS, Chiu CJ, Su LH (2007) Collateral damage of flomoxef therapy: in vivo development of porin deficiency and acquisition of $bla_{\text{DHA-1}}$ leading to ertapenem resistance in a clinical isolate of *Klebsiella pneumoniae* producing CTX-M-3 and SHV-5 β-lactamases. J Antimicrob Chemother 60:410–413

Liu JH, Deng YT, Zeng ZL, Gao JH, Chen L, Arakawa Y, Chen ZL (2008) Coprevalence of plasmid-mediated quinolone resistance determinants *QepA*, *Qnr*, and *AAC*(6′)*-Ib-cr* among 16S rRNA methylase RmtB-producing *Escherichia coli* isolates from pigs. Antimicrob Agents Chemother 52:2992–2993

Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, LF(Y (2016) Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis 16:161–168

Mahon CR, Lehman DC, Manuselis G (2007) Text book of diagnostic microbiology. 3rd ed. Philadelphia, PA, USA

Meacham KJ, Zhang L, Foxman B, Bauer RJ, Marrs CF (2003) Evaluation of genotyping large numbers of *Escherichia coli* isolates by enterobacterial repetitive intergenic consensus-PCR. J Clin Microbiol 41:5224–5226

Messai Y, Iabadene H, Benhassine T, Alouache S, Tazir M, Gautier V, Arlet G, Bakour R (2008) Prevalence and characterization of extended-spectrum β-lactamases in *Klebsiella pneumoniae* in Algiers hospitals (Algeria). Pathol Biol 56:319–325

Monstein HJ, Östholm-Balkhed Å, Nilsson MV, Nilsson M, Dornbusch K, Nilsson LE (2007) Multiplex PCR amplification assay for the detection of blaSHV, blaTEM and blaCTX-M genes in *Enterobacteriaceae*. Apmis 115:1400–1408

Munoz-Price LS, Poirel L, Bonomo RA, Schwaber MJ, Daikos GL, Cormican M, Cornaglia G, Garau J, Gniadkowski M, Hayden MK, Kumarasamy K (2013) Clinical epidemiology of the global expansion of *Klebsiella pneumoniae* carbapenemases. Lancet Infect Dis 13:785–796

Nobari S, Shahcheraghi F, Rahmati Ghezelgeh F, Valizadeh B (2014) Molecular characterization of carbapenem-resistant strains of *Klebsiella pneumoniae* isolated from Iranian patients: first identification of bla_{KPC} gene in Iran. Microb Drug Resist 20:285–293

Nordmann P, Cuzon G, Naas T (2009) The real threat of *Klebsiella pneumoniae* carbapenemase- producing bacteria. Lancet Infect Dis 9:228–236

Nordmann P, Poirel L, Walsh TR, Livermore DM (2011) The emerging NDM carbapenemases. Trends Microbiol 19:588–595

Pérez-Pérez FJ, Hanson ND (2002) Detection of plasmid-mediated AmpC β-lactamase genes in clinical isolates by using multiplex PCR. J Clin Microbiol 40:2153–2162

Poirel L, Walsh TR, Cuvillier V, Nordmann P (2011) Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis 70:119–123

Qin S, Zhou M, Zhang Q, Tao H, Ye Y, Chen H, Xu L, Xu H, Wang P, Feng X (2016) First identification of NDM-4-producing *Escherichia coli* ST410 in China. Emerg Microbes Infect 5:e118

Quan J, Li X, Chen Y, Jiang Y, Zhou Z, Zhang H, Sun L, Ruan Z, Feng Y, Akova M, Yu Y (2017) Prevalence of mcr-1 in *Escherichia coli* and *Klebsiella pneumoniae* recovered from bloodstream infections in China a multicenter longitudinal study. Lancet Infect Dis 7:400–410

Robicsek A, Strahilevitz J, Sahm DF, Jacoby GA, Hooper DC (2012) qnr prevalence in ceftazidime-resistant *Enterobacteriaceae* isolates from the United States. Antimicrob Agents Chemother 50: 2872–2874

Ruiz E, Sáenz Y, Zarazaga M, Rocha-Gracia R, Martínez-Martínez L, Arlet G, Torres C (2012) Qnr, aac(6′)-ib-cr and qepA genes in *Escherichia coli* and *Klebsiella* spp.: genetic environments and plasmid and chromosomal location. J Antimicrob Chemother 67:886–897

Samonis G, Maraki S, Karageorgopoulos DE, Vouloumanou EK, Falagas ME (2012) Synergy of fosfomycin with carbapenems, colistin, netilmicin, and tigecycline against multidrug-resistant *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa* clinical isolates. Eur J Clin Microbiol Infect Dis 31:695–701

Shibl A, Senok A, Memish Z (2012) Infectious diseases in the Arabian peninsula and Egypt. Clin Microbiol Infect 18:1068–1080

Shibl A, Al-Agamy M, Memish Z, Senok A, Khader SA, Assiri A (2013) The emergence of OXA-48- and NDM-1-positive *Klebsiella pneumoniae* in Riyadh, Saudi Arabia. Int J Infect Dis 17:e1130–e1133

Toleman MA, Walsh TR (2011) Combinatorial events of insertion sequences and ICE in Gram-negative bacteria. FEMS Microbiol Rev 35:912–935

Wachino JI, Yamane K, Kimura K, Shibata N, Suzuki S, Ike Y, Arakawa Y (2006) Mode of transposition and expression of 16S rRNA methyltransferase gene *rmtC* accompanied by ISEcp1. Antimicrob Agents Chemother 50:3212–3215

Wasfi R, Elkhatib WF, Ashour HM (2016) Molecular typing and virulence analysis of multidrug resistant *Klebsiella pneumoniae* clinical isolates recovered from Egyptian hospitals. Sci Rep 22:38929

Yun-Tae K, Kim T, Baik HS (2006) Characterization of extended spectrum β-lactamase genotype TEM, SHV, and CTX-M producing *Klebsiella pneumoniae* isolated from clinical specimens in Korea. J Microbiol Biotechnol 16:889–895