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Regular paper

Klebsiella pneumoniae: characteristics of carbapenem resistance and virulence factors*

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***Klebsiella pneumoniae*, known as a major threat to public health, is the most common factor of nosocomial and community acquired infections. In this study, 50 *K. pneumoniae* clinical specimens isolated from bronchial, urea, blood, catheter, rectal, bile, tracheal and wound cultures were collected. These isolates were identified and carbapenem resistance was determined *via* an automated system, CHROMagar Orientation and CHROMagar KPC. The carbapenemase gene regions (*blaIMP, blaVIM, blaOXA, blaNDM* and *blaKPC*) and presence of virulence factors (*magA, k_2A, rmpA, wabG, uge, allS, entB, ycfM, kpn, wcaG, fimH, mrkD, iutA, iroN, hly ve cnf-1*) of these isolates were determined by using Multiplex-PCR. The OXA-48 carbapenemase gene regions were determined in 33 of 50 *K. pneumoniae* strains. In addition, NDM-1 resistance in one, OXA-48 and NDM-1 resistance in four unusual *K. pneumoniae* isolates were detected. Virulence gene regions that were encountered among *K. pneumoniae* isolates were 88% wabG, 86% uge, 80% *ycfM* and 72% *entB*, related with capsule, capsule lipoprotein and external membrane protein, responsible for enterobactin production, respectively. Even though there was no significant difference between resistant and sensitive strains due to the virulence gene regions ($P \geq 0.05$), virulence factors in carbapenem resistant isolates were found to be more diverse. This study is important for both, to prevent the spread of carbapenem resistant infections and to plan for developing effective treatments. Moreover, this study is the first detailed study of the carbapenem resistance and virulence factors in *K. pneumoniae* strains.**

Key words: *Klebsiella pneumoniae*; multidrug carbapenem resistance; virulence factors

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INTRODUCTION

Carbapenem hydrolysing β-lactamases have been reported to be increasingly widespread. Ambler molecular class A (KPC), class B (VIM, IMP, NDM) and class D (OXA-48) types are the most often found among *Klebsiella pneumoniae* isolated during serious nosocomial infections (Nordmann *et al.*, 2011). Carbapenem resistant *K. pneumoniae* strains have been also recently reported in many countries in the world (Pfeifer *et al.*, 2012; Azap *et al.*, 2013; Balm *et al.*, 2013; Shibl *et al.*, 2013; Doi *et al.*, 2014).

Pathogenic *K. pneumoniae* strains have the potential to cause a wide variety of infectious diseases, including urinary tract, respiratory tract and blood infections (Podschun & Ullmann, 1998). Although these strains carry virulence associated genes, which may encode capsules (*magA, k_2A, wcaG*), hypermucoviscosity (*magA, rpmA*), adhesins (*fimH, mrkD, kpn*), lipopolysaccharides (*wabG, uge, ycfM*), iron acquisition systems (*iutA, iroN, entB*) and other virulence factors (*allS, hly, cnf-1*) that enable them to overcome host defenses (Hartman *et al.*, 2009; Yu *et al.*, 2008; Yu *et al.*, 2007; Yu *et al.*, 2006; Turton *et al.*, 2010; El Fertas-Aissani *et al.*, 2013; Mamlouk *et al.*, 2006; Guiral *et al.*, 2011; Sebghati *et al.*, 1998), it is not clear how these genes are associated with infection types or antibiotic resistance.

The aim of this study was to identify the genotypes of capsules, mucoviscosity, adhesins and other virulence factors of *K. pneumoniae* strains isolated from clinical specimens and to evaluate the association among potential virulence factors, carbapenem resistance and infection types.

METHODS

Bacterial strains and identification. 50 *Klebsiella pneumoniae* strains, obtained from clinical specimens including bronchial, urea, blood, catheter, rectal, bile, tracheal and wound infections, were collected from six different hospitals, in Ankara, Antalya, Istanbul, Kayseri, between 2010 and 2014, and were included in this study. Isolates were defined with the use of an automatized system (Vitek-32 System, bioMerrieux-France), CHROMagar Orientation (CHROMagar Company, Paris, France) and conventional phenotypic methods (classical biochemical properties, such as Gram staining, hemolysis of blood agar, string test, IMViC tests, lactose fermentation, ornithine decarboxylase and motility tests).

Antimicrobial susceptibility testing. Susceptibility to carbapenems was determined with Vitek-32 System and CHROMagar KPC (CHROMagar Company, Paris, France). Carbapenem resistant *K. pneumoniae* mucoid me-

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Abbreviations: allS, allantoin; cnf, cytotoxic necrotising factor; entB, enterobactin; hly, hemolysin; IMP, imipenem metallo-β-lactamase; IMViC, a group of individual tests; iroN, salmochelin; iutA, ferric aerobactin; fimH, type 1 fimbrial adhesin; k_2A, specific to K2 capsule serotype; KPC, *Klebsiella pneumoniae* carbapenemase; kpn, fimH like fimbrial adhesin; magA, mucoviscosity associated gene A; mrkD, type 3 fimbrial adhesion; NDM, New Delhi metallo-β-lactamase; OXA, oxacillinase; PCR, polymerase chain reaction; rmpA, regulator of mucoid phenotype A; uge, uridine diphosphate galacturonate 4-epimerase; VIM, verona integron-encoded metallo-β-lactamase; wabG, biosynthesis of the core lipopolysaccharide; wcaG, guanosine diphosphate-beta-L-fucose synthetase; ycfM, outer membrane lipoprotein.

Table 1. Carbapenemase gene regions (Poirel *et al.*, 2011).

Primer	Sequence* (5′-3′)	Gene	Product size (bp)
KPC-F	5′-CGTCTAGTTCTGCTGTCTTG-3′	bla_{KPC}	798
KPC-R	5′-CTTGTCATCCTTGTTAGGCG-3′		
NDM-1-F	5′-GGTTTGGCGATCTGGTTTTC-3′	bla_{NDM-1}	621
NDM-1-R	5′-CGGAATGGCTCATCACGATC-3′		
OXA-48-F	5′-GCGTGGTTAAGGATGAACAC-3′	bla-$_{OXA-48}$	438
OXA-48-R	5′-CATCAAGTTCAACCCAACCG-3′		
IMP-F	5′-GGAATAGAGTGGCTTAAYTCTC-3′	bla_{IMP}	232
IMP-R	5′-GGTTTAAYAAAACAACCACC-3′		
VIM-F	5′-GATGGTGTTTGGTCGCATA-3′	bla_{VIM}	390
VIM-R	5′-CGAATGCGCAGCACCAG-3′		

*Y=C ya da T

tallic blue colonies grew on CHROMagar KPC at 37ºC, 24 hours (Panagea *et al.*, 2011).

DNA isolation. Bacterial genomic and plasmid DNA was extracted from isolates by using NücleoSpin®Tissue and NücleoSpin®Plasmid (Macherey-Nagel, Germany), respectively.

Analysis of the carbapenemase gene regions. 2 µL of total DNA was subjected to multiplex PCR in a 50 µL reaction mixture. These reaction conditions were modified from Poirel *et al.*, 2011. The mix for the detection of *blaIMP, blaVIM* gene contains 1× PCR buffer (20 mM Tris HCl, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$, 0.1% Triton X-100), 0.05 mM dNTP, 2U Taq Polymerase (NEB, Beverly, MA), 50 µmol/L primers for five targets (NEB, Beverly, MA), described in Table 1. The mix for the detection of *blaKPC, blaNDM-1* and *blaOXA-48* was at the same concentrations. Amplification was carried out with the following thermal cycling conditions: 5 minutes of pre-denaturation at 95°C, followed by 35 cycles: 1 minute at 95°C, 1 minute at 52°C, 1 minute at 72°C and 10 minutes of final elongation at 72°C (Sensoquest Labcycler, Germany).

Analysis of the virulence gene regions. 2.5 µL of total DNA was subjected to multiplex PCR in a 50 µL reaction mixture. The mix for the detection of *magA, fimH, uge and iutA* genes contained 2X PCR buffer (40 mM Tris-HCl, 20 mM $(NH_4)_2SO_4$, 20 mM KCl, 4 mM $MgSO_4$, 0.2% Triton X-100), 0.2 mM dNTP, 2U Taq Polymerase (NEB, Beverly, MA) and 2.5 µmol/L primers (NEB, Beverly, MA) (Table 2). The mix for the detection of other groups (*wabG-rmpA-cnf1-ycfM, hly-iroN-k_2A-mrkD, kpn-allS-entB-wcaG)* was at the same concentrations. Amplification was carried out with the following thermal cycling conditions: 5 minutes of pre-denaturation at 95°C, followed by 30 cycles: 1 minute at 94°C, 1 minute at 58°C, 1 minute at 72°C and 10 minutes of final elongation at 72°C (Sensoquest Labcycler, Germany).

PCR products were analyzed by electrophoresis in a 1.8% agarose gel at 150 V for 2 h in 1×TBE (89 mM Tris, 89 mM Boric Acid and 2 mM EDTA) containing 0.05 mg/L ethidium bromide and using Gel Logic 200 Molecular Imaging System (Kodak; Rochester).

Data analysis. Clinical data were analyzed using "Minitab v17.1.0" software package for Windows. Fisher's Exact Test were performed. A difference was considered highly significant when $p \leq 0.05$.

RESULTS AND DISCUSSION

In this study, we have demonstrated that expressions of carbapenem resistance and presence of virulence genes in *K. pneumoniae* are weakly correlated in clinical specimens. Despite that, virulence factors in carbapenem resistant isolates were found to be more diverse.

Analysis of the carbapenemase gen regions

OXA-48 was first identified from *K.pneumoniae* in Turkey (Poirel *et al.*, 2004) and spread of OXA-48 producing *K.pneumoniae* in the European countries and Mediterranean area has been observed (Nordmann *et al.*, 2011). NDM-1 (New Delhi metallo-β-lactamase), one of the most clinically significant carbapenemase producer, was first reported in New Delhi, India (Yong *et al.*, 2009), followed by several case reports in United Kingdom, Pakistan and now worldwide (Dortet *et al.*, 2012). At the present time, co-producing NDM-1 and OXA group carbapenemases have been reported in Morocco (Abouddihaj *et al.*, 2012), Oman (Dortet *et al.*, 2012), Singapore (Balm *et al.*, 2013) and the United States (Doi *et al.*, 2014).

In this study, two different multiplex PCR reaction mixtures were defined for five resistance genes (*blaIMP, blaVIM, blaOXA, blaNDM and blaKPC*) and were used to study 50 *K. pneumoniae* strains. Among these isolates, only oxacillinase (OXA-48) gene was

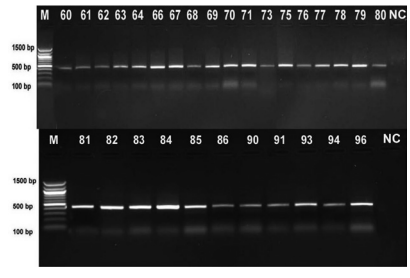

Figure 1. OXA-48 type carbapenemase of *Klebsiella pneumoniae* strains (60–96; *Klebsiella pneumoniae*, NC; Negative control, M; 100 bp DNA molecular marker).

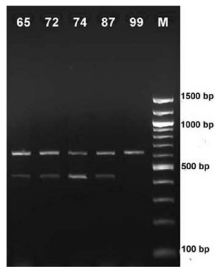

Figure 2. OXA-48 and NDM-1 type carbapenemase of *Klebsiella pneumoniae* strains (65, 72, 74, 87; co-producing OXA-48 and NDM-1, 99; NDM-1 type, M; 100 bp DNA molecular marker).

Table 2. Virulence gene regions of *K. pneumoniae*

Primer	Sequence (5′-3′)	Product size (bp)	
magA			
magA-F	5′- GGTGCTCTTTACATCATTGC-3′	1282	Yu *et al*., 2006
magA-R	5′-GCAATGGCCATTTGCGTTAG-3′		
k_2A			
k_2A-F	5′-CAACCATGGTGGTCGATTAG-3′	543	Yu *et al*., 2007
k_2A-R	5′-TGGTAGCCATATCCCTTTGG-3′		
rmpA			
rmpA-F	5′-ACTGGGCTACCTCTGCTTCA-3′	516	Yu *et al*., 2006 Turton *et al*., 2010
rmpA-R	5′-CTTGCATGAGCCATCTTTCA-3′		
wabG			
wabG-F	5′-ACCATCGGCCATTTGATAGA-3′	683	
wabG-R	5′-CGGACTGGCAGATCCATATC-3′		Yu *et al*., 2006
uge			
uge-F	5′-TCTTCACGCCTTCCTTCACT-3′	534	
uge-R	5′-GATCATCCGGTCTCCCTGTA-3′		
allS			
allS-F	5′-CCGAAACATTACGCACCTTT-3′	508	Yu *et al*., 2008
allS-R	5′-ATCACGAAGAGCCAGGTCAC-3′		
fimH			
fimH-F	5′-TGCTGCTGGGCTGGTCGATG-3′	688	Yu *et al*., 2008
fimH-R	5′-GGGAGGGTGACGGTGACATC-3′		
mrkD			
mrkD-F	5′-TTCTGCACAGCGGTCCC-3′	240	Sebghati *et al*., 1998
mrkD-R	5′-GATACCCGGCGTTTTCGTTAC-3′		
wcaG			
wcaG-F	5′-GGTTGGKTCAGCAATCGTA-3′	169	Turton *et al*., 2010
wcaG-R	5′-ACTATTCCGCCAACTTTTGC-3′		
kpn			
kpn-F	5′-GTATGACTCGGGGAAGATTA-3′	626	
kpn-R	5′-CAGAAGCAGCCACCACACG-3′		
ycfM			
ycf-F	5′-ATCAGCAGTCGGGTCAGC-3′	160	El Fertas-Aissani *et al*., 2013
ycf-R	5′-CTTCTCCAGCATTCAGCG-3′		
entB			
entB-F	5′-ATTTCCTCAACTTCTGGGGC-3′	371	
entB-R	5′-AGCATCGGTGGCGGTGGTCA-3′		
iutA			
iutA-F	5′-GGCTGGACATCATGGGAACTGG-3′	300	Mamlouk *et al*., 2006
iutA-R	5′- CGTCGGGAACGGGTAGAATCG-3′		
iroN			
iroN-F	5′-AAGTCAAAGCAGGGGTTGCCCG-3′	665	Guiral *et al*., 2011
iroN-R	5′-GACGCCGACATTAAGACGCAG-3′		
hly			
hly-F	5′-AACAAGGATAAGCACTGTTCTGGCT-3′	1177	
hly-R	5′-ACCATATAAGCGGTCATTCCCGTCA-3′		Mamlouk *et al*., 2006
cnf-1			
cnf-F	5′-AAGATGGAGTTTCCTATGCAGGAG-3′	498	
cnf-R	5′-CATTCAGAGTCCTGCCCTCATTATT-3′		

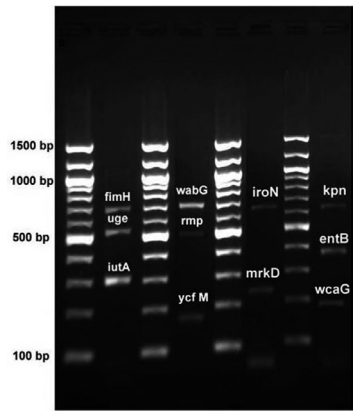

Figure 3. Determined virulence gene regions in *K. pneumoniae* strains

determined in 29 strains (58%); however, one (2%) of the isolates produced only New Delhi metallo-beta-lactamase 1 (NDM-1), 4 (8%) produced both NDM-1 and OXA-48 (Figs. 1 and 2). Similarly, carbapenem resistance in these strains was determined phenotypically, using CHROMagar KPC.

Co-producing NDM-1 and OXA-48 carbapenemases (one *K. pneumoniae* strain) in Turkey was reported by Alp *et al.*, (2013). In this study, four *K. pneumoniae* strains were found to produce both, NDM-1 and OXA-48. It is obvious that this resistance occurrence had increased in the last three years in Turkey.

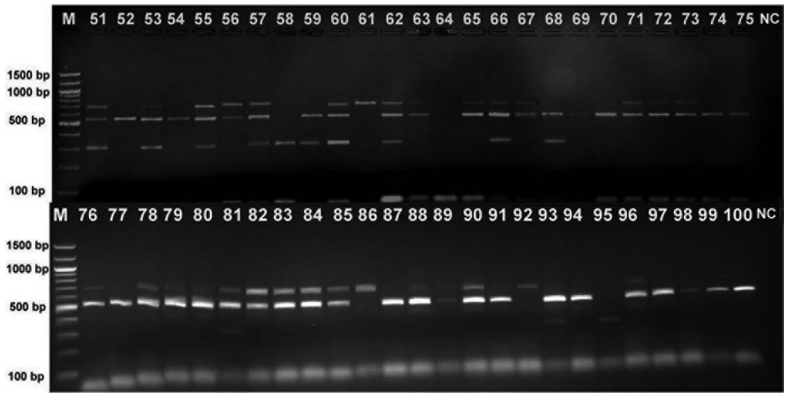

Figure 4. Determined *fimH, iutA* and *uge* gene regions in *K. pneumoniae* strains

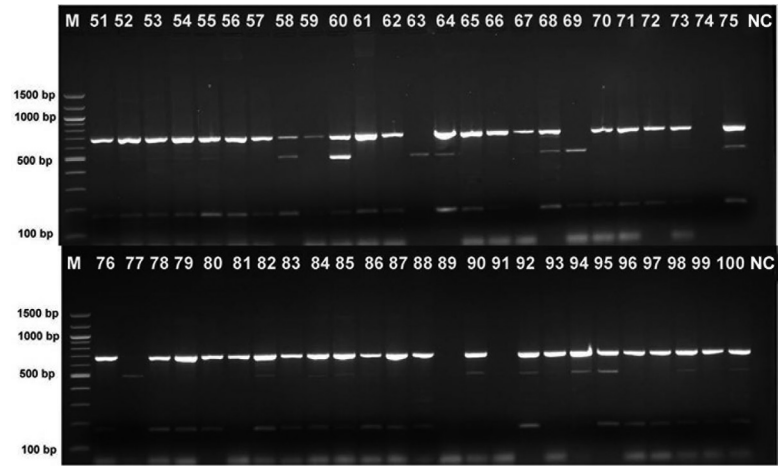

Figure 5. Determined *wabG, rmpA* and *ycfM* gene regions in *K. pneumoniae* strains

Analysis of the virulence gene regions

Four different multiplex PCR reaction mixtures were defined for 16 virulence genes (*magA, k$_2$A, rmpA, wabG, uge, allS, entB, ycfM, kpn, wcaG, fimH, mrkD, iutA, iro*N, *hly* ve *cnf-1*) and then were used to study 50 *K. pneumoniae* strains. The eleven determined virulence gene regions are shown in Fig 3. Band patterns of these strains, PCR positive for virulence genes, are given in Figs. 4–7.

There was no isolate detected containing the *magA, k$_2$A, cnf-1, hly* and *allS* genes. The *magA* (mucoviscosity-associated gene A and specific to K1 capsule serotype), *k$_2$A* (specific to K2 capsule serotype) and *allS* (associated with allantoin metabolism) genes play a decisive role in the pathogenesis of liver abscess (Fang *et al.*, 2004; Ku *et al.*, 2008). The absence of these genes indicated that there are no liver or abscesses specimens in this study. Study by Chou *et al.*, 2004 and Compain *et al.* (2014) also supports this situation. Besides this, the presence of *cnf-1, hly* and *allS* in *Klebsiella* are reported to be absent in other studies (Mamlouk *et al.*, 2006; Yu *et al.*, 2008).

Capsule associated genes (*wabG*, *uge* and *ycfM*) promote infection by resistance to phagocytosis (Cortés *et al.*, 2002). These genes were commonly found in *K. pneumoniae* isolates, they seem to be at the basis of pathogenicity of *K. pneumoniae.* In this study, virulence gene regions that we encountered among *K. pneumoniae* isolates were *wabG* (in 88% of isolates), *uge* (86%), *ycfM* (80%) and *entB* (72%), encoding the capsule, capsule lipoprotein, external membrane protein and enterobactin production, respectively (Fig. 8). These rates are consistent with previous studies reporting that *K. pneumoniae* clinical strains were producers of virulence factors (El Fertas-Aissani *et al.*, 2013).

According to distribution of virulence genes of *K. pneumoniae* strains, the most diversity in virulence was seen in urine and tracheal specimens, as shown in Fig. 9. This situation is closely related to the urinary tract infections and pneumonia caused frequently in humans. In addition, it was found that nine different virulence factors were present in rectal swab specimens which were recently isolated from pediatric colonization of patients.

The overall virulence factor productions among carbapenem resistant (n = 34) and carbapenem susceptible (n = 16) *K. pneumoniae* strains are shown in Table 3. These results indicate that there was no significant difference between resistant and sensitive strains due to the virulence gene regions ($P \geq 0.05$).

Forty virulence profiles were defined and when virulence and carbapenemase gene profiles were analyzed, virulence factors in carbapenem-resistant isolates were found to be more diverse, as shown in Table 4. Clinical *K. pneumoniae* strains express two types of fimbrial adhesins; type 1 and type 3 fimbriae (Podschun and Ullmann, 1998). While type 1 fimbriae, encoding *fimH,* play an important role in urinary tract infections caused by these strains, type 3 fimbriae, encoding *mrkD*, promote biofilm devel-

Table 3. Distribution of carbapenem resistant and susceptible *K. pneumoniae* strains

Virulence factors	Carbapenem resistant *K. pneumoniae* (n=34) (%)	Carbapenem susceptible *K. pneumoniae* (n=16) (%)	*P* value
magA	0	0	–
k_2A	0	0	–
cnf-1	0	0	–
hly	0	0	–
allS	0	0	–
wcaG	1 (3)	0	1.000
iroN	2 (6)	0	1.000
mrkD	22 (65)	7 (44)	0.222
iutA	6 (18)	7 (44)	0.082
rmpA	12 (35)	5 (31)	1.000
kpn	14 (41)	11 (69)	0.122
fimH	22 (65)	6 (38)	0.126
entB	22 (65)	14 (88)	0.175
ycfM	25 (74)	15 (94)	0.138
uge	31 (91)	12 (75)	0.190
wabG	29 (85)	15 (94)	0.650

opment (Struve *et al.*, 2009). Besides it, siderophores encoding *entB, iutA* and *iroN*, are iron binding proteins and they also promote biofilm formation (May and Okabe, 2011; El Fertas-Aissani *et al.*, 2013). In this study, total fimbrial adhesins (*fimH, mrkD* and *kpn*) were observed in 42 strains (84%) and siderophores (*entB, iutA* and *iroN*) were observed in 40 strains (80%) (Table 4). This situation shows that these virulence factors are important for *Klebsiella* pathogenicity.

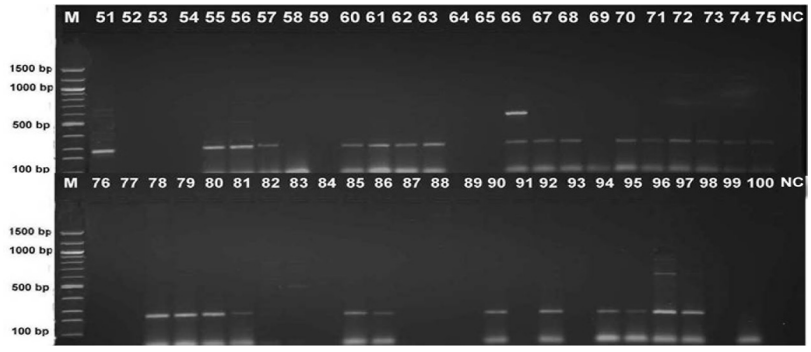

Figure 6. Determined *mrkD* and *iroN* gene regions in *K. pneumoniae* strains

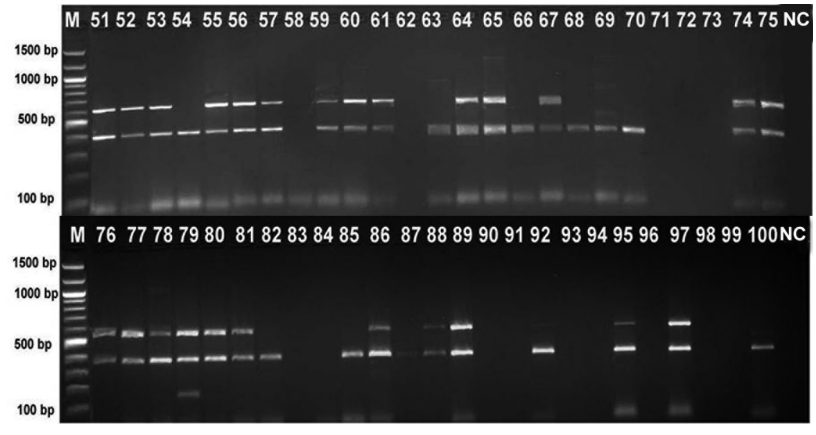

Figure 7. Determined *kpn, entB* and *wcaG* gene regions in *K. pneumoniae strains*

It is interesting to note that the carbapenem resistance strain no. 91 has only one virulence factor (*uge*), but carbapenem susceptible strains no. 53, 55, 56, 57 and 95 have at least seven virulence factors. In addition, however, 55—57 and 56–78 groups have the same virulence factors, although the carbapenem resistance or clinical source of the strains in the same group are different. This situation shows that there is no correlation among carbapenem resistance, virulence factors and infection types.

It is known that virulence factors and antibiotic resistance are generally considered to play a significant role in bacterial pathogenesis (Beceiro *et al.*, 2013). Many studies have reported that virulence factors are associated with antibiotic resistance in pathogenic bacteria (Arısoy *et al.*, 2008; El Fertas-Aissani *et al.*, 2013), however, the present study indicates that there is no significant correlation among virulence factors, carbapenem resistance and infections types. Recently, a few studies have

indicated that quorum sensing affects these mechanisms (Yang *et al.*, 2012; Wang *et al.*, 2013). Consequently, the study presented here demonstrated that virulence factors, antibiotic resistance and quorum sensing molecules should be considered in a collective manner in further studies on bacterial pathogenesis for developing effective treatments.

Acknowledgements

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Table 4. Carbapenem resistance and virulence gene profiles of *K. pneumoniae* strains

Strains	Clinical sources	Carbapenem resistance	Virulence gene profiles
91	Urine	OXA-48	*uge*
93	Urine	OXA-48	*iutA, uge, wabG*
80	Rectal	OXA-48	*mrkD, entB, ycfM*
97	Urine	susceptible	
54	Urine	susceptible	*entB, uge, wabG, ycfM*
87	Urine	NDM-1 and OXA-48	
58	Wound	susceptible	*iutA, wabG, ycfM, rmpA*
89	Urine	susceptible	*fimH, kpn, entB, uge*
83	Bronchial	OXA-48	*fimH, uge, wabG, ycfM*
99	Blood	NDM-1	
69	Wound	OXA-48	*entB, uge, ycfM, rmpA*
77	Tracheal	OXA-48	*kpn, entB, uge, rmpA*
94	Urine	OXA-48	*ycfM, uge, wabG, rmpA*
98	Urine	susceptible	*uge, wabG, ycfM, rmpA*
52	Urine	susceptible	*kpn, entB, uge, wabG, ycfM*
88	Urine	susceptible	
63	Urine	OXA-48	*fimH, mrkD, entB, uge, rmpA*
74	Wound	NDM-1 and OXA-48	*fimH, mrkD, kpn, entB, uge*
64	Wound	OXA-48	*kpn, entB, wabG, ycfM, rmpA*
71	Tracheal	OXA-48	*fimH, mrkD, uge, wabG, ycfM*
72	Urine	NDM-1 and OXA-48	
73	Urine	OXA-48	
70	Catheter	OXA-48	*mrkD, entB, uge, wabG, ycfM*
100	Urine	susceptible	*entB, uge, wabG, ycfM, rmpA*
84	Tracheal	OXA-48	*fimH, uge, wabG, ycfM, rmpA*
90	Urine	OXA-48	*fimH, mrkD, uge, wabG, rmpA*
51	Urine	susceptible	*mrkD, kpn, entB, iutA, wabG, ycM,*
59	Wound	susceptible	*kpn, entB, iutA, uge, wabG, ycfM*

61	Bile	OXA-48	*fimH, mrkD, kpn, entB, wabG, ycfM*
86	Wound	OXA-48	
62	Tracheal	OXA-48	*fimH, mrkD, iutA, uge, wabG, ycfM*
65	Urine	NDM-1 and OXA-48	*fimH, kpn, entB, uge, wabG, ycfM*
76	Catheter	OXA-48	
67	Urine	OXA-48	*fimH, mrkD, kpn, entB, uge, wabG*
96	Urine	OXA-48	*fimH, mrkD, iroN, uge, wabG, ycfM*
82	Rectal	OXA-48	*fimH, entB, uge, wabG, ycfM, rmpA*
92	Urine	susceptible	*fimH, mrkD, entB, wabG, ycfM, rmpA*
53	Urine	susceptible	*fimH, kpn, entB, iutA, uge, wabG, ycfM*
56	Tracheal	susceptible	*fimH, mrkD, kpn, entB, uge, wabG, ycfM*
78	Wound	OXA-48	
81	Blood	OXA-48	*fimH, mrkD, kpn, entB, iutA, uge, wabG*
68	Urine	OXA-48	*mrkD, entB, iutA, uge, wabG, ycfM, rmpA*
95	Wound	susceptible	*mrkD, kpn, entB, iutA, wabG, ycfM, rpmA*
79	Rectal	OXA-48	*mrkD, kpn, entB, uge, wabG, ycfM, wcaG*
75	Tracheal	OXA-48	*mrkD, kpn, entB, uge, wabG, ycfM, rmpA*
85	Urine	OXA-48	*fimH, mrkD, entB, uge, wabG, ycfM, rmpA*
55	Urine	susceptible	*fimH, mrkD, kpn, entB, iutA, uge, wabG, ycfM*
57	Tracheal	susceptible	
66	Tracheal	OXA-48	*fimH, mrkD, entB, iutA, iroN, uge, wabG, ycfM*
60	Urine	OXA-48	*fimH, mrkD, kpn, entB, iutA, uge, wabG, ycfM, rmpA*

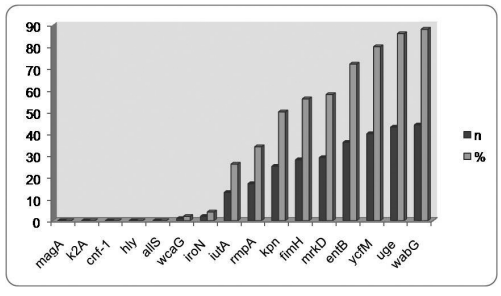

Figure 8. Distribution of virulence genes of *K. pneumoniae* strains. "n" is the number of isolates that were found to possess a given gene; "%" represents n as the percenatge of the 50 strains studied.

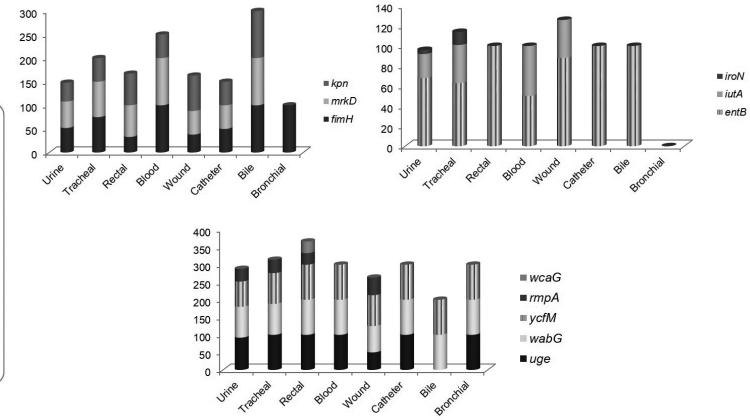

Figure 9. Percent distribution of virulence genes in different clinical sources (*urine; 25, tracheal; 8, rectal; 3, blood; 2, wound; 8, catheter; 2, bile; 1, bronchial; 1 strain*)

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