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Short Communication

Carbapenem-resistant *Klebsiella pneumoniae* isolates from Egypt containing bla_{NDM-1} on IncR plasmids and its association with *rmtF*

Doaa Gamal [a,b,1,*], Marta Fernández-Martínez [a,1], Dalia Salem [b], Inas El-Defrawy [b], Laura Álvarez Montes [a], Alain A. Ocampo-Sosa [a], Luis Martínez-Martínez [a,c]

[a] University Hospital Marqués de Valdecilla–IDIVAL, Santander, Spain
[b] Microbiology Department, Theodor Bilharz Research Institute (TBRI), Cairo, Egypt
[c] Molecular Biology Department, School of Medicine, University of Cantabria, Santander, Spain

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SUMMARY

Objectives: The aim of this study was to characterize carbapenem-resistant *Klebsiella pneumoniae* (CRKP) isolates recovered from clinical specimens at a tertiary care hospital in Egypt over a period of 15 months.
Methods: Eight CRKP isolates were included in this study. The minimum inhibitory concentrations of different antibiotics were determined by broth microdilution and Etest methods. Multilocus sequence typing was performed. Antibiotic resistance genes were assessed by PCR and DNA sequencing. Plasmid analysis was done by S1 nuclease digestion of whole genomic DNA followed by pulsed-field gel electrophoresis (S1-PFGE).
Result: Eight carbapenem-resistant NDM-1-producing *K. pneumoniae* isolates of three different sequence types (ST) were identified (ST147, ST11, and ST17), in which bla_{NDM-1} was carried by either IncR or untypeable plasmids. Seven out of the eight isolates also contained the *rmtF* methylase gene.
Conclusion: This study describes the occurrence of IncR plasmids carrying bla_{NDM-1} and *rmtF* in Egypt, raising concerns regarding this type of replicon and its role in the transmission of these resistance determinants.

New Delhi metallo-β-lactamase (NDM)-producing *Klebsiella pneumoniae* isolates have spread globally, causing infections with a significant and high mortality rate.[1] Over a period of 15 months, from September 2013 to December 2014, a total of 157 *Klebsiella spp* isolates were recovered from different clinical specimens processed at the microbiology laboratory of Theodor Bilharz Research Institute (TBRI), a tertiary care hospital in Egypt. Thirteen of them were found to be resistant to imipenem or meropenem by disk diffusion and Vitek2 system (bioMérieux, Marcy L'Etoile, France). Eight of these isolates were available for this study.

Minimum inhibitory concentrations (MICs) of a set of antibiotics were determined by standardized broth microdilution method following the guidelines of the Clinical and Laboratory Standards Institute (CLSI).[2] Those of ertapenem and meropenem were also performed by Etest strip method (bioMérieux, Marcy L'Etoile, France) (Table 1). Data were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints,[3] except for nalidixic acid, which was categorized according to CLSI standards.[2] One isolate (K4) was susceptible to gentamicin, ciprofloxacin, and nalidixic acid (Table 1). The remaining seven isolates were resistant to cephalosporins, carbapenems, quinolones, and aminoglycosides. All isolates retained susceptibility to both colistin and tigecycline. Discrepancies between the broth microdilution and Etest results were noted regarding carbapenems (Table 1). Such discordance between the two methods has been reported previously in VIM-1-producing *K. pneumoniae*.[4]

PCR was performed, followed by sequencing for the genes coding for extended-spectrum β-lactamases (bla_{SHV}, bla_{TEM}, bla_{CTX-M}), plasmid-mediated AmpC β-lactamases, carbapenemases (bla_{KPC}, bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{OXA-48}), plasmid-mediated quinolone resistance (*qnrA*, *qnrB*, *qnrS*), aminoglycoside-modifying enzymes (*aac(3)-Ia*, *aac(3)-IIa*, *aac(3)-IVa*, *aac(6′)-Ib ant(2″)-Ia*, *aph(3′)-Ia*, *aph(3′)-IIa*, *aph-(3′)-VIa*), and methyltransferases (*npmA*, *armA*, *rmtB*, *rmtC*, and *rmtF*).[5–8]

* Corresponding author.
E-mail address: doaagamal@gmail.com (D. Gamal).
[1] Doaa Gamal and Marta Fernández-Martínez contributed equally to this article.

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Table 1
Mechanisms of resistance to selected antimicrobial agents in *Klebsiella pneumoniae* isolates and derived transformants and transconjugants

PFGE/ST	Isolate	MIC (μg/ml)														Plasmid replicon	Resistance determinants
		CAZ	CTX	IMP	MEM	MEM (Etest)	ERT (Etest)	AK	GEN	TOB	CIP	NAL	SXT	TIG	COL		
A/147	K1	>128	>128	>128	>128	>32	>32	>128	>128	>128	>128	>128	>128	2	0.125	colE, R	*NDM-1*, *CTX-M-15*, *SHV-11*, *aac(3)-IIa*, *aph(3′)-Ia*, *aac(6′)-Ib-cr*, *rmtF*, *qnrB*
	TF K1	>128	>128	4	8	1	1	16	64	32	0.125	4	>128	≤0.06	≤0.06	R	*NDM-1*, *CTX-M-15*, *aac(3)-IIa*, *aph(3′)-Ia*, *aac(6′)-Ib-cr*, *qnrB*
	K6	>128	>128	16	128	2	6[a]	>128	>128	>128	>128	>128	>128	2	0.25	colE, R	*NDM-1*, *CTX-M-15*, *SHV-11*, *aac(3)-IIa*, *aph(3′)-Ia*, *aac(6′)-Ib-cr*, *rmtF*, *qnrB*
	K7	>128	>128	16	16	2	6[a]	>128	>128	>128	>128	>128	>128	1	≤0.06	colE, R	*NDM-1*, *CTX-M-15*, *SHV-11*, *aac(3)-IIa*, *aph(3′)-Ia*, *aac(6′)-Ib-cr*, *rmtF*, *qnrB*
	K9	>128	>128	16	32	2	6	>128	>128	>128	>128	>128	>128	1	0.125	colE, R	*NDM-1*, *CTX-M-15*, *SHV-11*, *aac(3)-IIa*, *aph(3′)-Ia*, *aac(6′)-Ib-cr*, *rmtF*, *qnrB*
	TF K9	>128	>128	4	8	1	1.5[a]	8	64	32	0.25	4	>128	≤0.06	0.125	R	*NDM-1*, *CTX-M-15*, *aac(3)-IIa*, *aph(3′)-Ia*, *aac(6′)-Ib-cr*, *qnrB*
	K10	>128	>128	>128	>128	>32	>32	>128	>128	>128	>128	>128	>128	2	0.125	colE, R	*NDM-1*, *CTX-M-15*, *SHV-11*, *aac(3)-IIa*, *aph(3′)-Ia*, *aac(6′)-Ib-cr*, *rmtF*, *qnrB*
B/17	K4	>128	>128	4	8	2	2	128	0.5	8	0.5	8	>128	0.5	0.125	colE, R, L/M	*NDM-1*, *CTX-M-14*, *SHV-11*, *SHV-12*, *TEM-1*, *aph(3′)VIa*, *qnrS*
	TF K4	>128	>128	4	8	1	0.75[a]	8	0.5	4	0.125	4	>128	≤0.06	≤0.06	R	*NDM-1*, *SHV-12*, *aph(3′) VIa*, *qnrS*
	TC K4	8	>128	≤0.06	≤0.06	0.023	0.06	128	1	1	≤0.06	2	32	≤0.06	≤0.06	L/M	*CTX-M-14*, *aph(3′)VIa*
C/11	K5	>128	>128	128	>128	>32	>32	>128	>128	>128	>128	>128	>128	0.25	0.25	colE, R, repF	*NDM-1*, *CTX-M-15*, *SHV-11*, *aph(3′)-Ia*, *aac(6′)-Ib*, *rmtF*, *qnrB*
	TF K5	>128	>128	4	4	0.75	1	>128	>128	>128	≤0.06	2	16	≤0.06	≤0.06	Untypeable	*NDM-1*, *aac(6′)-Ib*, *rmtF*, *qnrB*
	K11	>128	>128	4	64	4[a]	>32	>128	>128	>128	128	>128	>128	1	0.125	colE, R, repF	*NDM-1*, *CTX-M-15*, *SHV-11*, *aph(3′)-Ia*, *aac(6′)-Ib*, *rmtF*, *qnrB*
	TC K11	>128	>128	8	8	0.5[a]	0.75[a]	>128	>128	>128	0.125	8	>128	≤0.06	≤0.06	Untypeable	*NDM-1*, *aac(6′)-Ib*, *rmtF*, *qnrB*
	E. coli Top10	0.25	≤0.06	0.25	≤0.06	0.016	0.002	2	0.5	0.5	≤0.06	2	4	0.125	≤0.06	NA	NA
	E. coli J53	0.5	≤0.06	0.25	≤0.06	0.012	0.002	4	2	1	≤0.06	8	>128	≤0.06	≤0.06	NA	NA

PFGE, pulsed-field gel electrophoresis; ST, sequence type; MIC, minimum inhibitory concentration; CAZ, ceftazidime; CTX, cefotaxime; IMP, imipenem; MEM, meropenem; ERT, ertapenem; AK, amikacin; GN, gentamicin; TOB, tobramycin; CIP, ciprofloxacin; NAL, nalidixic acid; SXT, trimethoprim–sulfamethoxazole; TIG, tigecycline; COL, colistin; TF, transformed cell; TC, transconjugant; NA, not applicable.

[a] Indicates the presence of microcolonies that increase in size and number over time.