

Epidemic IncX3 plasmids spreading carbapenemase genes in the United Arab Emirates and worldwide

This article was published in the following Dove Press journal:
Infection and Drug Resistance

Shaimaa F Mouftah^{1,*}

Tibor Pál^{1,*}

Dania Darwish¹

Akela Ghazawi¹

Laura Villa²

Alessandra Carattoli³

Ágnes Sonnevend^{1,4}

¹Department of Medical Microbiology and Immunology, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates;

²Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy;

³Department of Molecular Microbiology, University of Rome La Sapienza, Rome, Italy;

⁴Department of Medical Microbiology and Immunology, Medical School, University of Pécs, Pécs, Hungary

*These authors contributed equally to this work

Purpose: Plasmids of the incompatibility group X type 3 (IncX3) were described carrying various carbapenemase genes in carbapenemase-producing *Enterobacteriaceae* (CPE) worldwide and in the United Arab Emirates (UAE), as well. To understand the driving force behind the emergence of such plasmids in the UAE, the relationship between IncX3 plasmids encountered locally and globally was investigated.

Methods: CPE strains isolated in the UAE during 2009–2014 were screened by X3 PCR-based replicon typing. The clonal relationship of CPE carrying IncX3 plasmids was determined by multi-locus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE). Complete sequence of selected IncX3 plasmids was determined. Phylogenetic relationship between the carbapenemase carrying IncX3 plasmids from the UAE and of those reported worldwide was established by comparing the plasmid backbones.

Results: 10.2% of the 295 CPE tested were identified to carry IncX3 plasmids: 13 *Escherichia coli*, 13 *Klebsiella pneumoniae*, two *Enterobacter cloacae*, one *Citrobacter freundii* and one *Morganella morganii* isolate, respectively. Most of them were non-clonal; with small clusters of triplets and pairs of *E. coli* and *K. pneumoniae*, and a cluster of five *K. pneumoniae* ST11 exhibiting >90% similar PFGE patterns, respectively. The 30 isolates harbored either *bla*_{NDM-1}, *bla*_{NDM-4}, *bla*_{NDM-5}, *bla*_{NDM-7}, *bla*_{OXA-181} or *bla*_{KPC-2} carbapenemase genes on IncX3 plasmids. Phylogenetic analysis of the backbone region of IncX3 plasmids carrying various beta-lactamase genes from the UAE (n=23) and that of North-America, Europe, Asia and Australia (n=35) revealed three clusters based on the carbapenemase genes carried: plasmids harboring *bla*_{OXA-181} and *bla*_{NDM-5} formed two distinct groups, whereas backbones of plasmids with *bla*_{NDM-1}, *bla*_{NDM-4} and *bla*_{NDM-7} clustered together. Each cluster contained plasmids of diverse geographical origin.

Conclusion: The findings suggest that different carbapenemase gene carrying IncX3 plasmids encountered in the UAE do not evolve locally, rather are subtypes of this epidemic plasmid emerging in this country due to international transfer.

Keywords: Enterobacterales, carbapenemase genes, IncX3 plasmid, Middle-East

Introduction

Due to the limited therapeutic options remaining to treat these infections, carbapenemase-producing *Enterobacteriaceae* (CPE) are increasingly important human pathogens associated with high mortality.^{1,2} Their spread is driven by two major forces: clonal dissemination of a few successful CPE lineages, and horizontal transfer of carbapenemase genes often located on epidemic plasmids spreading in different bacterial species, sources and countries.^{2–5} Plasmids of the incompatibility

Correspondence: Ágnes Sonnevend
Department of Medical Microbiology and Immunology, College of Medicine and Health Sciences, United Arab Emirates University, P.O. Box 17666, Al Ain, United Arab Emirates
Tel +971 3 713 7481
Fax +971 3 767 1966
Email asonnevend@gmail.com

group (Inc) X defined as X3 type (IncX3)⁶ have been reported worldwide in *Enterobacteriales*, associated with *bla*_{SHV-12} extended-spectrum beta-lactamase (ESBL), *bla*_{KPC-2, -3}, *bla*_{NDM-1, -4, -5, -7} and *bla*_{OXA-181} carbapenemase genes.^{7–14} IncX3 plasmids were reported to disseminate a variety of *bla*_{NDM} genes in humans, in animals and in the environment particularly in South East Asia; including China, Hong Kong, South Korea, Myanmar, Vietnam and the Indian Subcontinent.^{12–20}

The Middle-East is considered an endemic region for CPE, with the dominance of class D OXA-48-like, and class B NDM carbapenemases, with sporadic occurrence of class A KPC-2, and class B VIM-4 enzymes.^{1,2,7–10} In the Arabian Peninsula autochthonous, clonal transmission has been implicated as the main driving force in the emergence of CPE,¹ but plasmid-mediated dissemination of *bla*_{VIM-4} has also been documented in the region.²¹ Furthermore, sporadic isolates carrying *bla*_{NDM-1}, *bla*_{NDM-7} and *bla*_{KPC-2} on IncX3 plasmids were identified in the United Arab Emirates (UAE).^{22–24} However, the role of this type of plasmid in the dissemination of CPE, and its possible local evolution have not been systematically studied. Here, we present the comparisons of the complete sequences of IncX3 plasmids carrying various carbapenemase genes encountered in the UAE, and evaluate their relatedness to similar episomes identified worldwide.

Materials and methods

Bacterial strains

Altogether 334 non-repeat carbapenem-resistant *Enterobacteriales* (CRE) strains were tested. They were isolated between April 2009 and December 2014 in 12 hospitals of the UAE and submitted to the Department of Medical Microbiology and Immunology, UAE University, without any patient identifiers, to identify the carbapenemases produced. Strains were stored at -80°C in Tryptic Soy Broth (MAST, Merseyside, UK) containing 20% glycerol. This collection included 90 isolates described earlier in^{1,22–24} and further 246 CRE isolated between May 2013 and December 2014 in six governmental hospitals of Abu Dhabi Emirate.

Detection of carbapenemase genes and screening for the IncX3 replicon

The presence of the *bla*_{NDM}, *bla*_{OXA-48-like}, *bla*_{VIM}, *bla*_{IMP}, *bla*_{KPC} carbapenemase genes, and that of *bla*_{SHV} were detected as described.^{25–27} The specific alleles of beta-

lactamase genes were determined by direct sequencing of the respective amplicons with the Big Dye Cycle Terminator V.3.1 (Applied Biosystems) using the 3130X Genetic Analyzer (Applied Biosystems). A replicase-specific PCR was used to screen strains for the presence of IncX3 plasmids.⁶

Antibiotic susceptibility assays and phenotypic detection of carbapenemase production

The antibiotic susceptibility of carbapenemase-producing IncX3 plasmid carrying clinical isolates and their derivatives to cefotaxime, ceftazidime, aztreonam, ertapenem, meropenem, imipenem, ciprofloxacin, gentamicin, amikacin, trimethoprim/sulfamethoxazole, tetracycline and colistin was tested by broth microdilution, while fosfomycin and tigecycline susceptibilities were assessed by agar dilution.²⁸ CLSI clinical breakpoints were used for interpretation for the majority of antibiotics.²⁸ Results for colistin, tigecycline and fosfomycin were interpreted by the EUCAST criteria.²⁹ Carbapenemase production was assessed phenotypically by the CIM test.³⁰

Molecular typing

Carbapenemase-producing IncX3 positive *K. pneumoniae*, *E. coli* and *E. cloacae* isolates were typed using pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST).^{31–34}

Characterization of the carbapenemase gene-bearing IncX3 plasmids

Plasmids were isolated by the alkaline lysis method, and detected as described in²³ using *E. coli* 39R861 as plasmids' molecular size standards. Southern blotting of the plasmid electrophoresis gel, and hybridization with IncX3 and the respective carbapenemase gene probes was used to prove the localization of carbapenemase genes on IncX3 plasmid.²³

The sequence of each IncX3 plasmids carried by different ST and/or PFGE profiles were further investigated. In case of multiple strains exhibiting the same ST and PFGE profile, plasmids were chosen from strains representing each unique plasmid profiles and/or coding for each distinct carbapenemases.

In mating out assays, a sodium-azide resistant derivative of rifampicin-resistant *E. coli* J53 (J53_{RAZ}) was used as recipient. Transconjugants were selected on Tryptic Soy Agar containing 8 mg/L⁻¹ ceftazidime and 100 mg/L⁻¹

sodium-azide, or in case of OXA-181 producing clinical isolates on 0.5 mg/L^{-1} ertapenem and 100 mg/L^{-1} sodium-azide.³⁵ When transconjugants were not obtained, the IncX3 plasmids were transformed into competent *E. coli* DH5 α or *E. coli* GM2163.³⁵ For complete plasmid sequencing, plasmid DNA was purified from single plasmid containing *E. coli* transconjugant or transformant using the Plasmid Maxi Prep kit (Qiagen, Hilden, Germany). The complete sequence of the plasmids was established by next-generation sequencing either by using the 454-Genome Sequencer FLX procedure (Roche Diagnostic, Monza, Milan, Italy) or, commercially, on the Illumina MiSeq platform (performed at the CCIB DNA Core Facility in Massachusetts General Hospital, Cambridge, MA, USA). The gaps between contigs assembled were closed by PCR and direct sequencing of the amplicons. The complete plasmid sequences were assembled with Clone Manager v9.0 (Sci-Ed Software, Cary, NC, US), annotated using Geneious R11.0.4 (Biomatters Ltd., Auckland, New Zealand) and Sequin (<http://www.ncbi.nlm.nih.gov/Sequin>), and submitted to GenBank (Accession numbers are shown in Table 2).

For comparison, all complete sequences of IncX3 plasmids carrying carbapenemase genes available in GenBank up to January 2019 were downloaded. If identical plasmid backbone sequences, carrying the same carbapenemase gene were identified in multiple isolates from the same country, only one was selected randomly for the phylogenetic analysis.

Plasmid backbones of the UAE IncX3 plasmid sequences and those retrieved from GenBank were aligned by ClustalW, and the evolutionary history was inferred by the Jukes-Cantor genetic distance model with 500x bootstrapping using Geneious R11.0.4 (Biomatters Ltd., Auckland, New Zealand).

Results

Characteristics of strains carrying IncX3 plasmids

Of the 334 isolates screened, 295 were positive for at least one carbapenemase gene by PCR. The remaining 39 were

negative by PCR for the five common carbapenemase genes tested, and they were carbapenemase non-producers by the CIM test. The distribution of the 32 IncX3 plasmid carrying isolates among strains with various carbapenem resistance genes is shown in Table 1. The IncX3 positive CPE isolates were variably resistant to 3rd generation cephalosporins, aztreonam, aminoglycosides, ciprofloxacin, co-trimoxazole, tetracycline, tigecycline, colistin and fosfomycin (shown in Table S1). The characteristics of the 30 CPE isolates, in which at least one carbapenemase gene was located on an IncX3 plasmid, are shown in Table 2. Altogether five species of *Enterobacterales* were identified. One *Citrobacter freundii*, one *Morganella morganii* and one *Enterobacter cloacae* carried *bla*_{NDM-1} on IncX3 plasmid, and a further *Enterobacter cloacae* harbored IncX3-borne *bla*_{NDM-4}.

The 13 *E. coli* carried either *bla*_{NDM-1}, *bla*_{NDM-5}, *bla*_{NDM-7} or *bla*_{OXA-181} on IncX3 plasmids. They exhibited limited clonality; a triplet and two pairs of isolates formed PFGE clusters with $\geq 90\%$ pattern similarity, respectively (Figure S1A). The 13 *E. coli* belonged to 8 different sequence types (Table 2). The 13 *K. pneumoniae* were less heterogeneous: five *K. pneumoniae* ST11 carrying IncX3-borne *bla*_{NDM-1} exhibited $\geq 90\%$ similar PFGE patterns, three NDM-1 and OXA-48 co-producing *K. pneumoniae* ST1318, with *bla*_{NDM-1} being located on IncX3 plasmid, also clustered by PFGE, and the two KPC-2 producer *K. pneumoniae* ST14 were indistinguishable by PFGE (Figure S1B). The further three *K. pneumoniae* were of different sequence types; two of them carried *bla*_{OXA-181}, and one had *bla*_{NDM-5} on an IncX3 plasmid. This latter isolate, a *K. pneumoniae* ST307, co-produced NDM-5 and OXA-162, but *bla*_{OXA-162} was not located on the IncX3 plasmid (Table 2).

Characteristics of IncX3 plasmids carrying carbapenemase genes

Altogether 21 IncX3 plasmids were selected for further analysis. Single plasmid-bearing derivatives obtained by conjugation or by transformation (Table 2) showed

Table 1 Distribution of IncX3 plasmid carrying isolates among strains expressing different carbapenem resistance mechanisms

n	All	NDM	OXA-48-like	NDM and OXA-48-like	VIM	KPC	All carbapenemase producer	Carbapenemase non-producer
IncX3 PCR positive	334 32 (9.6%)	89 18 (20%)	126 6 (4.8%)	75 4 (5.3%)	3 0	2 2 (100%)	295 30 (10.2%)	39 2 (5.1%)

Table 2 Characteristics of carbapenemase producing *Enterobacteriaceae* harboring IncX3 type plasmids with carbapenemase genes

Isolate			Plasmid							Ref.		
Name*	Date of isolation	Hospital	Specimen	Species	Carbapenemase produced	MLST	C/Cm/nonC	Name	Size (bp)	Resistance gene(s)	GenBank Acc. No	
ABC133	12/14/2012	TH	Sputum	<i>E. coli</i>	NDM-7	ST4108	nonC	pABC133-NDM	37070	<i>bla</i> _{NDM-7}	KX214671	24
ABC239	8/15/2013	RH	Urine	<i>E. coli</i>	OXA-181	ST410	nonC	pABC239-OXA-181	51479	<i>bla</i> _{OXA-181} + <i>qnrS</i> /	MK412916	This study
ABC264	6/9/2014	TH	Unknown	<i>E. coli</i>	OXA-181	ST410	nonC	pABC264-OXA-181	51479	<i>bla</i> _{OXA-181} + <i>qnrS</i> /	MK412917	This study
ABC356	8/8/2014	MH	Urine	<i>E. coli</i>	OXA-181	ST410	Cm	pABC356-OXA-181	51479	<i>bla</i> _{OXA-181} + <i>qnrS</i> /	MK412918	This study
ABC381	11/4/2014	AAH	Rectal swab	<i>E. coli</i>	OXA-181	ST167	nonC	pABC381-OXA-181	51479	<i>bla</i> _{OXA-181} + <i>qnrS</i> /	MK412919	This study
ABC218	12/25/2012	RH	Wound	<i>E. coli</i>	NDM-7	ST167	C	pABC218-NDM	34403	<i>bla</i> _{NDM-7}	KX214670	24
ABC233	7/21/2013	RH	Urine	<i>E. coli</i>	NDM-5	ST167	Cm	pABC233-NDM-5	46161	<i>bla</i> _{NDM-5}	MK372390	This study
ABC384	11/5/2014	AAH	Urine	<i>E. coli</i>	NDM-5	ST1284	C	pABC384-NDM-5	46161	<i>bla</i> _{NDM-5}	MK372389	This study
ABC54	1/2/2011	TH	Urine	<i>E. coli</i>	NDM-1	ST2206	C	pABC54-NDM-1	53023	<i>bla</i> _{NDM-1} + <i>bla</i> _{SHV-12}	MK372382	This study
BC-13-836	9/24/2013	TH	Blood	<i>E. coli</i>	NDM-1	ST446	C	pBC836-NDM-1	52565	<i>bla</i> _{NDM-1} + <i>bla</i> _{SHV-11}	MK372387	This study
ABC280	7/15/2014	TH	Urine	<i>E. coli</i>	NDM-5	ST448	C	pABC280-NDM5	35502	<i>bla</i> _{NDM-5}	MK372392	This study
ABC286	8/15/2014	TH	Blood	<i>E. coli</i>	NDM-5	ST448	NT	NT	NT	NT	NT	This study
ABC268	6/11/2014	AAH	Urine	<i>E. coli</i>	NDM-5	ST2083	Cm	pABC268-NDM-5	45232	<i>bla</i> _{NDM-5}	MK372391	This study
ABC40	10/27/2009	TH	Wound	<i>E. cloacae</i>	NDM-1	ST417	Cm	pABC40-NDM-1	54035	<i>bla</i> _{NDM-1} + <i>bla</i> _{SHV-12}	MK372380	This study
ABC302	2/26/2014	MH	Urine	<i>E. cloacae</i>	NDM-4	ST200	C	ABC302-NDM-4	49402	<i>bla</i> _{NDM-4}	MK372388	This study
BC-13-947	7/11/2013	TH	Blood	<i>K. pneumoniae</i>	OXA-181	ST2095	nonC	pBC947-OXA-181	51479	<i>bla</i> _{OXA-181} + <i>qnrS</i> /	MK412920	This study
ABC260	3/31/2014	TH	Rectal swab	<i>K. pneumoniae</i>	OXA-181	ST3545	nonC	pABC260-OXA-181	51480	<i>bla</i> _{OXA-181} + <i>qnrS</i> /	MK412915	This study
ABC369	9/23/2014	TH	Abdominal fluid	<i>K. pneumoniae</i>	NDM-5+ OXA-162	ST307	Cm	pABC369-NDM-5	45252	<i>bla</i> _{NDM-5}	MK372393	This study
ABC137	1/14/2013	MH	Wound	<i>K. pneumoniae</i>	NDM-1+ OXA-48	ST1318	Cm	pABC137-NDM-1	53022	<i>bla</i> _{NDM-1} + <i>bla</i> _{SHV-12}	MK372384	This study
ABC141	4/20/2013	MH	Unknown	<i>K. pneumoniae</i>	NDM-1+ OXA-48	ST1318	NT	NT	NT	NT	NT	This study
ABC155	6/5/2013	SKMC	Blood	<i>K. pneumoniae</i>	NDM-1+ OXA-48	ST1318	NT	NT	NT	NT	NT	This study
ABC220	10/5/2012	RH	Wound	<i>K. pneumoniae</i>	KPC-2	ST14	C [#]	pABC220-KPC-2	46900	<i>bla</i> _{KPC-2}	MK412914	This study
ABC224	3/17/2013	RH	Sputum	<i>K. pneumoniae</i>	KPC-2	ST14	C [#]	NT	NT	NT	NT	This study
ABC52	9/19/2010	TH	Sputum	<i>K. pneumoniae</i>	NDM-1	ST11	C	pABC52-NDM-1	52565	<i>bla</i> _{NDM-1} + <i>bla</i> _{SHV-12}	MK372381	This study
ABC53	9/19/2010	TH	Sputum	<i>K. pneumoniae</i>	NDM-1	ST11	NT	NT	NT	NT	NT	This study
BC680	7/18/2012	TH	Blood	<i>K. pneumoniae</i>	NDM-1	ST11	NT	NT	NT	NT	NT	This study

(Continued)

Table 2 (Continued).

Isolate			Plasmid							Ref.		
Name*	Date of isolation	Hospital	Specimen	Species	Carbapenemase produced	MLST	C/Cm/nonC	Name	Size (bp)	Resistance gene(s)	GenBank Acc. No	
BC700 BC-13-817	7/24/2012	TH	Blood	<i>K. pneumoniae</i>	NDM-1	ST11	C	pBC700-NDM-1	52565	<i>bla</i> _{NDM-1} + <i>bla</i> _{SHV-11}	MK372386	This study
	9/17/2013	TH	Blood	<i>K. pneumoniae</i>	NDM-1	ST11	NT	NT	NT	NT	NT	This study
ABC80	5/8/2011	TH	Urine	<i>Citrobacter freundii</i>	NDM-1	NT	Cm	pABC80-NDM-1	53023	<i>bla</i> _{NDM-1} + <i>bla</i> _{SHV-12}	MK372383	This study
ABC140	3/25/2013	MH	Perianal swab	<i>Morganella morganii</i>	NDM-1	NA	nonC	pABC140-NDM-1	52591	<i>bla</i> _{NDM-1}	MK372385	This study

Notes: *Strain names with bold indicate those from which the IncX3 plasmid was fully sequenced in this study.

Abbreviations: MLST, Multi-Locus Sequence Typing; ST, sequence type; NA, not applicable; NT, not tested; C, conjugative; Cm, conjugative with transconjugants having multiple plasmids; C^{tr}, conjugative, but cannot stably maintained in *E. coli* J53_RAZ⁺; nonC, not self-transmissible.

varying degrees of non-susceptibility to carbapenems and to 3rd generation cephalosporins and were susceptible to non-beta lactam antibiotics (Table S1).

Complete DNA sequences of the 21 plasmids were obtained and compared to two IncX3 plasmids carrying *bla*_{NDM-7} (pABC133-NDM and pABC218-NDM), previously described from the UAE²⁴ (Table 2 and Figure 1).

In pABC220-KPC-2, the *bla*_{KPC-2} gene was located on a Tn4401b transposon, and no further resistance gene was carried by this plasmid.

The six *bla*_{OXA-181} carrying plasmids were >99% similar to each other, and all of them harbored the *bla*_{OXA-181} and a *qnrS1* quinolone resistance gene in a composite transposon bracketed by IS26.

The genetic load region of the eight *bla*_{NDM-1} carrying plasmids was flanked by IS26 and Tn3. The immediate genetic surrounding of the *bla*_{NDM-1} between an ISCR27 and a truncated IS*Aba125* was identical in all eight plasmids. The IS26 bracketed composite transposon upstream of ISCR27 either carried *bla*_{SHV-11} (n=2), or *bla*_{SHV-12} (n=5), or contained a truncated Tn3 transposase (pABC140-NDM-1). The genetic surroundings of *bla*_{NDM-4}, *bla*_{NDM-5} and *bla*_{NDM-7} between IS26 and IS5 were identical.

Although the genetic load regions were different in plasmids having various classes of carbapenemases, the plasmid backbones were highly similar with the notable absence of *hns*, and variable presence of complete or truncated *topB* and *ATPase* genes in pABC280-NDM-5, pABC218-NDM and pABC133-NDM (Figure 1).

Phylogenesis of the carbapenemase gene-bearing IncX3 plasmids

As pABC218-NDM, despite a large deletion in the conserved region, demonstrated to be self-conjugative and sufficiently stable, a 24905 bp long region coding for its replication, partitioning and transfer (from position 1286 to 26190 in GenBank Acc. No. KX214670) was used in the phylogenetic analysis. This backbone region was extracted from all complete IncX3 plasmid sequences from the UAE, and from the complete sequence of 35 IncX3 plasmids from different geographical regions downloaded from GenBank (listed in Table S2).

The Neighbor-Joining tree of the 58 IncX3 plasmid backbone sequences (Figure 2) showed three distinct clades. The first contained *bla*_{NDM-1}, *bla*_{NDM-4} and *bla*_{NDM-7} carrying plasmids from the UAE and plasmids

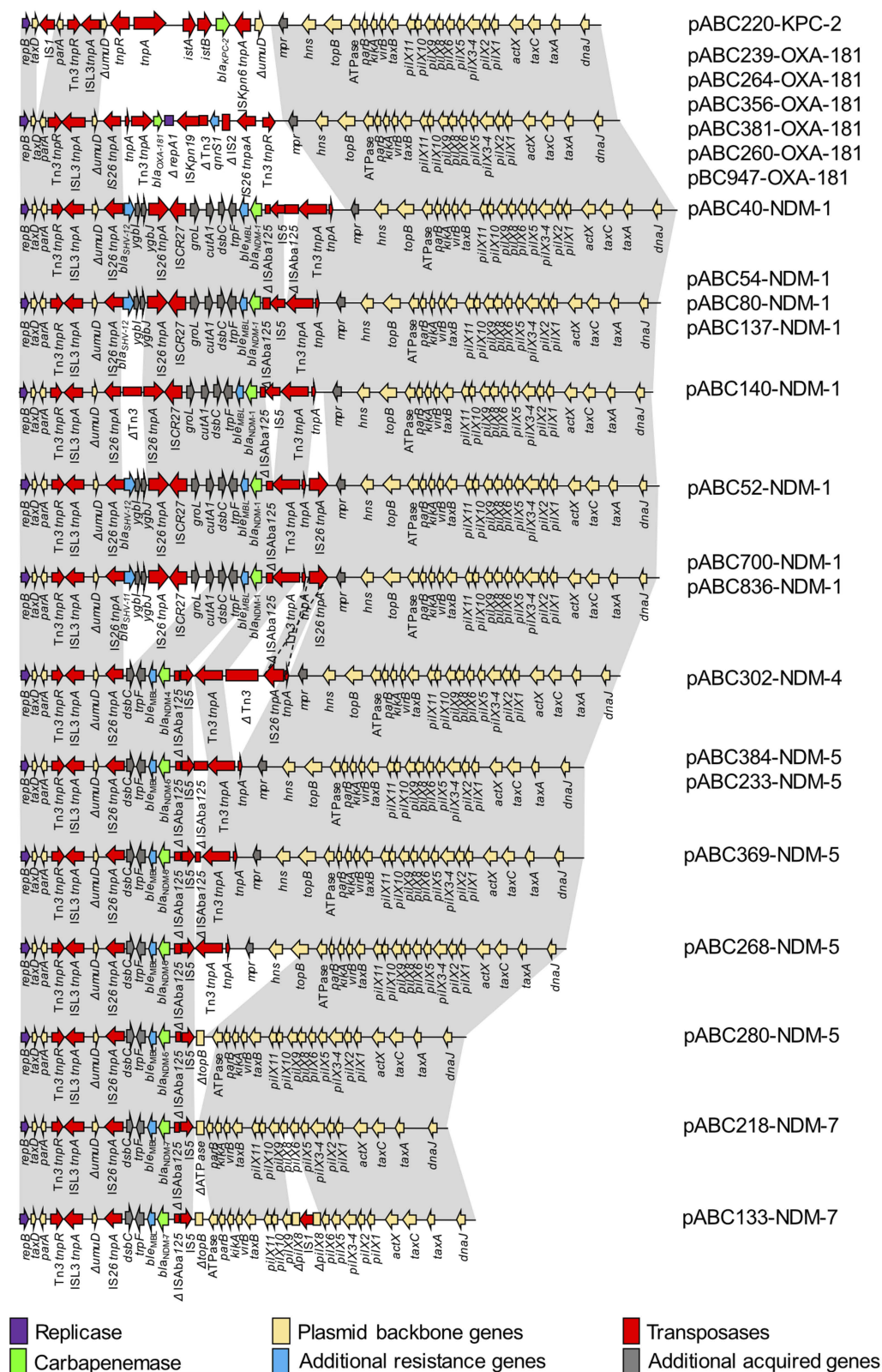


Figure 1 Comparison of IncX3 plasmids from the United Arab Emirates carrying various carbapenemases.

Notes: Grey shades represent regions with ≥99% similarity.

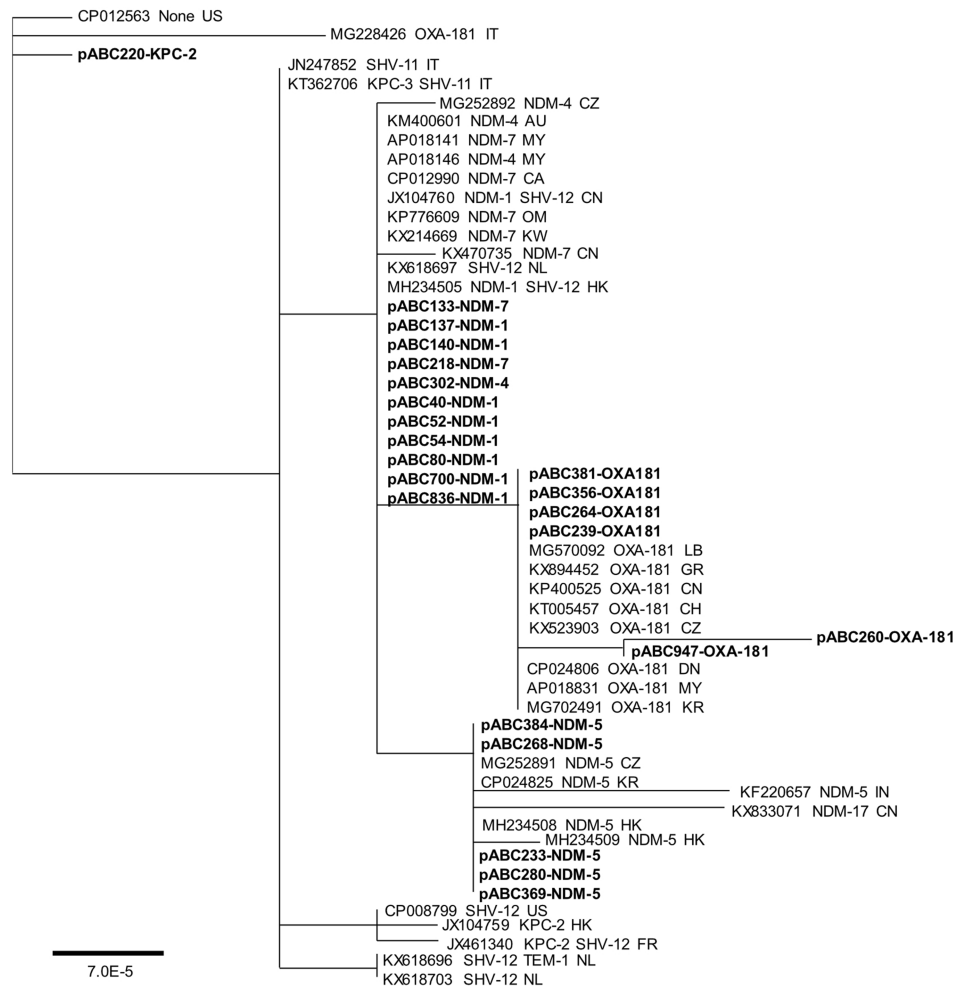


Figure 2 Phylogenetic tree of backbone sequences of IncX3 plasmids from various geographical area.

Notes: The sequences were aligned using ClustalV, and the Neighbor-joining tree was constructed using the Jukes-Cantor genetic distance model with 500 bootstrap replicates. All positions containing gaps and missing data were eliminated. There was a total of 24,868 positions in the final dataset. Plasmid names printed in bold represent IncX3 plasmids from the UAE, for plasmids retrieved from GenBank the accession number, the beta-lactamase gene carried, and the country of isolation is shown.

Abbreviations: CA, Canada; CH, Switzerland; CN, China; CZ, Czech Republic; DN, Denmark; FR, France; GR, Germany; HK, Hong Kong; IN, India; IT, Italy; KR, South Korea; KW, Kuwait; LB, Lebanon; MY, Myanmar; NL, the Netherlands; OM, Oman; US, United States of America.

carrying similar carbapenemase genes of other geographical regions, and a *bla*_{SHV-12} carrying plasmid from The Netherlands. The second one included *bla*_{NDM-5} carrying plasmids, and the third clade clustered *bla*_{OXA-181} carrying IncX3 plasmids originating from various parts of the world with a single outlier of *bla*_{OXA-181} carrying IncX3 plasmid (MG228426) from Italy only. Conversely, plasmids harboring *bla*_{KPC} were distinct from each other.

Discussion

Our data showed that in CRE isolated in 12 hospitals of the UAE, the overall prevalence of IncX3 plasmids was 9.6%, and in NDM-producer as high as 20%. Importantly, in the 30 CPE, the carbapenemase gene (or one of them in the double carbapenemase producers) was located on an IncX3 type plasmid.

This is a prevalence substantially higher than the one reported in human fluoroquinolone or cefotaxime resistant *E. coli* isolates,⁷ but considerably lower compared to a report on CRE from Hong Kong (30.3%).¹²

The CRE isolates carrying IncX3 with a carbapenemase gene were quite diverse. They belonged to five different species of *Enterobacterales* (*K. pneumoniae*, *E. coli*, *E. cloacae*, *C. freundii* and *M. morgannii*). Similar, or even higher diversity of hosts of carbapenemase bearing IncX3 plasmids has been noted in South-East Asian countries.^{12,16}

The majority of CRE isolates carrying carbapenemase-encoding IncX3 plasmids were unrelated. However, PFGE clustering of five *K. pneumoniae* ST11 harboring *bla*_{NDM-1} on IncX3 plasmids, all isolated in the same hospital, suggested clonal dissemination. Interestingly, the two plasmids

sequenced from these five isolates carried different *bla*_{SHV} alleles: *bla*_{SHV-12} and *bla*_{SHV-11} differing in three nucleotides, otherwise being 100% identical to each other. The combination of carbapenemase carrying IncX3 plasmid and the *K. pneumoniae* ST11 clone, both considered to have epidemic potential,⁵ is especially worrisome.

Interestingly, two *K. pneumoniae* ST14, which were described earlier in,²² carried *bla*_{KPC-2}, although this clone was found to be the most common NDM- and OXA-48-like producer *K. pneumoniae* clone in Dubai in a later period, when no KPC-producing isolates were encountered.³⁶

A member of another high-risk *K. pneumoniae* clone, ST307, was also encountered possessing *bla*_{OXA-162} and an IncX3 plasmid-borne *bla*_{NDM-5}. To the best of our knowledge, *bla*_{OXA-162} has not previously been associated with this clone. It is noteworthy that the same ST had been reported earlier from the UAE to carry *bla*_{NDM-1} on an IncHI1B plasmid and *bla*_{OXA-162} on IncL/M plasmid.³⁵ While that isolate did not harbor an IncX3 plasmid, it was recovered in the same hospital as the current one with the IncX3 *bla*_{NDM-5} plasmid, and was also co-harboring a *bla*_{OXA-162}. Therefore, the possibility of local acquisition of *bla*_{NDM-5} carrying IncX3 plasmid cannot be excluded.

A cluster of three OXA-181 producing *E. coli* ST410 harboring the carbapenemase on IncX3 plasmids was also encountered. Recently, it was established that this sequence type of *E. coli* is also an emerging high-risk clone.³⁷ The three *E. coli* ST167 isolates carried three different carbapenemases: *bla*_{NDM-5}, *bla*_{NDM-7} and *bla*_{OXA-181}, all located on IncX3 plasmids (Table 2). This clone is considered to be an epidemic NDM-5-producing *E. coli* clone in China³⁸ and was shown to carry IncX3 plasmid-borne *bla*_{NDM-5} in the Czech Republic, too.³⁹ It was also reported to harbor *bla*_{NDM-7} on IncX3 plasmid from France⁴⁰ and India.⁴¹ However, *E. coli* ST167 with *bla*_{OXA-181} carrying IncX3 plasmid has not been encountered yet, although a single locus variant of ST167 was reported to carry this carbapenemase gene from São Tomé and Príncipe.⁴²

It has been suggested that the wide dissemination of IncX3 plasmids is due to its highly efficient conjugal transfer, contributing to its spread within clinical settings, as well as in the environment.^{12,16} Based on our studies we cannot comment on these observations, since several of our plasmids co-transferred with other episomes, and some were non-conjugative, despite genes for conjugal transfer were apparently present and intact in all but one plasmid of our collection (pABC133-NDM described in²⁴).

Similarly, we cannot comment on the role of the environmental dissemination suggested earlier,^{12,24,43} as the current study included human isolates only.

Since many, but not all, carbapenemase carrying IncX3 plasmids resided in international high-risk clones of *Enterobacteriaceae*, we compared the conserved regions of plasmids from the UAE to the ones reported earlier from various countries (Table S2) to evaluate whether these plasmids occur in the UAE as a result of local evolution, or rather as a consequence of international transfer. The analysis identified clades exhibiting good correlation with the carbapenemase genes carried (Figure 2), ie close phylogenetic relationship of IncX3 plasmids harboring *bla*_{NDM-1}, *bla*_{NDM-4} and *bla*_{NDM-7} from the UAE and from different countries of the Middle-East, Asia, Europe and North-America was observed. On the other hand, *bla*_{NDM-5} carrying plasmids from the UAE, Czech Republic, China, Hong Kong, India and South Korea formed a distinct clade. Previously, based on the high degree of synteny among the complete NDM-IncX3 plasmid sequences, the evolution of *bla*_{NDM} alleles within the IncX3 plasmid was suggested.¹² Our findings partially support this hypothesis with the notion that certain *bla*_{NDM} alleles, notably that of NDM-5, are located on plasmids with a more distantly related backbone, suggestive of multiple uptakes of *bla*_{NDM} genes by these plasmids.

*bla*_{OXA-181} carrying IncX3 plasmids encountered in the UAE, as well as in Lebanon, Germany, Denmark, Czech Republic, Switzerland, China, South Korea and Myanmar formed another distinct clade with a single outlier (MG228426) from Italy, only. The KPC-IncX3 plasmids were phylogenetically heterogeneous: while two *bla*_{KPC-2} harboring plasmids from Hong Kong and from France mapped relatively close (JX104759 and JX461340), the backbone of the plasmid coding for the same allele from of the UAE (pABC220-KPC-2) and that of an Italian plasmid carrying *bla*_{KPC-3} (KT362706) were distant.

Conclusion

Phylogenetic analysis, clustering backbones of IncX3 plasmids of diverse geographical origin based on the carbapenemase gene carried, suggests that these plasmids disseminate across the continents. Consequently, the emergence of different carbapenemase carrying IncX3 plasmids in the UAE is likely not the result of local evolution, but due to the international transfer of such plasmids. Moreover, finding of high-risk *K. pneumoniae* and *E. coli* clones in the UAE, harboring these plasmids, warrants further studies to better understand

the role of the epidemic plasmids and clones in the emergence and spread of CPE in the country highly exposed to international travel and trade.

Acknowledgments

This work was supported by grants from the College of Medicine and Health Sciences, United Arab Emirates University 31M251 and United Arab Emirates University UPAR-31M235 awarded to ÁS.

Disclosure

The authors report no conflicts of interest in this work.

References

- Sonnevend Á, Ghazawi AA, Hashmey R, et al. Characterization of carbapenem-resistant *Enterobacteriaceae* with high rate of autochthonous transmission in the Arabian Peninsula. *PLoS One*. 2015;10:e0131372. doi:10.1371/journal.pone.0131372
- van Duin D, Doi Y. The global epidemiology of carbapenemase-producing *Enterobacteriaceae*. *Virulence*. 2017;8:460–469. doi:10.1080/21505594.2016.1222343
- Poirel L, Bonnin RA, Nordmann P. Genetic features of the widespread plasmid coding for the carbapenemase OXA-48. *Antimicrob Agents Chemother*. 2012;56:559–562. doi:10.1128/AAC.05289-11
- Woodford N, Johnson AP. Global spread of antibiotic resistance: the example of New Delhi metallo- β -lactamase (NDM)-mediated carbapenem resistance. *J Med Microbiol*. 2013;62:499–513. doi:10.1099/jmm.0.052555-0
- Navon-Venezia S, Kondratyeva K, Carattoli A. *Klebsiella pneumoniae*: a major worldwide source and shuttle for antibiotic resistance. *FEMS Microbiol Rev*. 2017;41:252–275. doi:10.1093/femsre/fux013
- Johnson TJ, Bielak EM, Fortini D, et al. Expansion of the IncX plasmid family for improved identification and typing of novel plasmids in drug-resistant *Enterobacteriaceae*. *Plasmid*. 2012;68:43–50. doi:10.1016/j.plasmid.2012.03.001
- Dobiasova H, Dolejska M. Prevalence and diversity of IncX plasmids carrying fluoroquinolone and β -lactam resistance genes in *Escherichia coli* originating from diverse sources and geographical areas. *J Antimicrob Chemother*. 2016;71:2118–2124. doi:10.1093/jac/dkw144
- García-Fernández A, Villa L, Carta C, et al. *Klebsiella pneumoniae* ST258 producing KPC-3 identified in Italy carries novel plasmids and OmpK36/OmpK35 porin variants. *Antimicrob Agents Chemother*. 2012;56:2143–2145. doi:10.1128/AAC.06446-11
- Feng Y, Wu W, Liu Y, et al. First report of OXA-181-producing *Escherichia coli* in China and characterization of the isolate using whole-genome sequencing. *Antimicrob Agents Chemother*. 2015;59:5022–5025. doi:10.1128/AAC.00442-15
- Mataseje LF, Boyd DA, Fuller J, et al. Characterization of OXA-48-like carbapenemase producers in Canada, 2011–14. *J Antimicrob Chemother*. 2018;73:626–633. doi:10.1093/jac/dkx462
- Liakopoulos A, van der Goot J, Bossers A, et al. Genomic and functional characterisation of IncX3 plasmids encoding bla_{SHV-12} in *Escherichia coli* from human and animal origin. *Sci Rep*. 2018;8:1–13. doi:10.1038/s41598-018-26073-5
- Wang Y, Tong M-K-K, Chow K-H-H, et al. Occurrence of highly conjugative IncX3 epidemic plasmid carrying bla_{NDM} in *Enterobacteriaceae* isolates in geographically widespread areas. *Front Microbiol*. 2018;9:1–8.
- Ho PL, Li Z, Lo WU, et al. Identification and characterization of a novel incompatibility group X3 plasmid carrying bla_{NDM-1} in *Enterobacteriaceae* isolates with epidemiological links to multiple geographical areas in China. *Emerg Microbes Infect*. 2012;1:e39. doi:10.1038/emi.2012.33
- Lin C-H, Ho P-L, Cheng V-C-C, et al. Molecular characterization of an atypical IncX3 plasmid pKPC-NY79 carrying bla_{KPC-2} in a *Klebsiella pneumoniae*. *Curr Microbiol*. 2013;67:493–498. doi:10.1007/s00284-013-0398-2
- Wu W, Feng Y, Tang G, et al. NDM Metallo- β -Lactamases and their bacterial producers in health care settings. *Clin Microbiol Rev*. 2019;32:e00115–18. doi:10.1128/CMR.00115-18
- Sugawara Y, Akeda Y, Sakamoto N, et al. Genetic characterization of bla_{NDM}-harboring plasmids in carbapenem-resistant *Escherichia coli* from Myanmar. *PLoS One*. 2017;12:e0184720. doi:10.1371/journal.pone.0184720
- Qin S, Cheng J, Wang P, et al. Early emergence of OXA-181-producing *Escherichia coli* ST410 in China. *J Glob Antimicrob Resist*. 2018;15:215–218. doi:10.1016/j.jgar.2018.06.017
- Zhu B, Ying C, Xu H, et al. Coexistence of NDM-1-producing *Escherichia coli* and *Citrobacter freundii* in the same patient. *J Glob Antimicrob Resist*. 2018;15:79–81. doi:10.1016/j.jgar.2018.04.013
- Xiang R, Zhang AY, Ye XL, et al. Various sequence types of *Enterobacteriaceae* isolated from commercial chicken farms in China and carrying the bla_{NDM-5} gene. *Antimicrob Agents Chemother*. 2018;62:e00779–18. doi:10.1128/AAC.00779-18
- Li X, Fu Y, Shen M, et al. Dissemination of bla_{NDM-5} gene via an IncX3-type plasmid among non-clonal *Escherichia coli* in China. *Antimicrob Resist Infect Control*. 2018;7:59. doi:10.1186/s13756-018-0349-6
- Sonnevend Á, Yahfoufi N, Ghazawi A, et al. Contribution of horizontal gene transfer to the emergence of VIM-4 carbapenemase producer *Enterobacteriaceae* in Kuwait. *Infect Drug Resist*. 2017;10:469–478. doi:10.2147/IDR.S149321
- Sonnevend A, Ghazawi A, Darwish D, et al. Characterization of KPC-type carbapenemase-producing *Klebsiella pneumoniae* strains isolated in the Arabian Peninsula. *J Antimicrob Chemother*. 2014;70:1592–1593. doi:10.1093/jac/dku445
- Sonnevend A, Al Baloushi A, Ghazawi A, et al. Emergence and spread of NDM-1 producer *Enterobacteriaceae* with contribution of IncX3 plasmids in the United Arab Emirates. *J Med Microbiol*. 2013;62:1044–1050. doi:10.1099/jmm.0.059014-0
- Pál T, Ghazawi A, Darwish D, et al. Characterization of NDM-7 carbapenemase-producing *Escherichia coli* isolates in the Arabian Peninsula. *Microb Drug Resist*. 2017;23:871–878. doi:10.1089/mdr.2016.0069
- Ghazawi A, Sonnevend A, Bonnin RA, et al. NDM-2 carbapenemase-producing *Acinetobacter baumannii* in the United Arab Emirates. *Clin Microbiol Infect*. 2012;18:E34–E36. doi:10.1111/j.1469-0691.2011.03726.x
- Poirel L, Walsh TR, Cuvillier V, et al. Multiplex PCR for detection of acquired carbapenemase genes. *Diagn Microbiol Infect Dis*. 2011;70:119–123. doi:10.1016/j.diagmicrobio.2010.12.002
- Cao V, Lambert T, Nhu DQ, et al. Distribution of extended-spectrum β -lactamases in clinical isolates of *Enterobacteriaceae* in Vietnam. *Antimicrob. Agents Chemother*. 2002;46:3739–3743. doi:10.1128/AAC.46.12.3739-3743.2002
- Clinical Laboratory Standards Institute Performance Standard for Antimicrobial Susceptibility Testing – M100. 29th; 2019. Available from: <http://em100.edaptivedocs.net/GetDoc.aspx?doc=CLSIM100ED29:2019&scope=user>. Accessed March 15, 2019.
- EUCAST: clinical breakpoints and dosing of antibiotics. Eur Comm Antimicrob Susceptibility Testing clin breakpoint for bacteria 2019. Available from: http://www.eucast.org/clinical_breakpoints/. Accessed March 15, 2019.

30. van der Zwaluw K, de Haan A, Pluister GN, et al. The Carbapenem inactivation method (CIM), a simple and low-cost alternative for the carba NP test to assess phenotypic carbapenemase activity in gram-negative rods. *PLoS One*. 2015;10:e0123690. doi:10.1371/journal.pone.0123690
31. Gautam RK. Rapid pulsed-field gel electrophoresis protocol for typing of *Escherichia coli* O157: h7 and other gram-negative organisms in 1 day. *J Clin Microbiol*. 1997;35:2977–2980.
32. Diancourt L, Passet V, Verhoef J, et al. Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. *J Clin Microbiol*. 2005;43:4178–4182. doi:10.1128/JCM.43.8.4178-4182.2005
33. Wirth T, Falush D, Lan R, et al. Sex and virulence in *Escherichia coli*: an evolutionary perspective. *Mol Microbiol*. 2006;60:1136–1151. doi:10.1111/j.1365-2958.2006.05172.x
34. Miyoshi-Akiyama T, Hayakawa K, Ohmagari N, et al. Multilocus sequence typing (MLST) for characterization of *Enterobacter cloacae*. *PLoS One*. 2013;8:e66358. doi:10.1371/journal.pone.0066358
35. Al-Baloushi AE, Pál T, Ghazawi A, et al. Genetic support of carbapenemases in double carbapenemase producer *Klebsiella pneumoniae* isolated in the Arabian Peninsula. *Acta Microbiol Immunol Hung*. 2018;65:135–150. doi:10.1556/030.65.2018.005
36. Moubareck CA, Mouftah SF, Pál T, et al. Clonal emergence of *Klebsiella pneumoniae* ST14 co-producing OXA-48-type and NDM carbapenemases with high rate of colistin resistance in Dubai, United Arab Emirates. *Int J Antimicrob Agents*. 2018;52:90–95. doi:10.1016/j.ijantimicag.2018.03.003
37. Roer L, Overballe-Petersen S, Hansen F, et al. *Escherichia coli* sequence type 410 is causing new International high-risk clones. *MSphere*. 2018;3:e00337–18. doi:10.1128/mSphere.00337-18
38. Xu L, Wang P, Cheng J, et al. Characterization of a novel *bla*_{NDM-5} - harboring IncFII plasmid and an *mcr-1*-bearing IncI2 plasmid in a single *Escherichia coli* ST167 clinical isolate. *Infect Drug Resist*. 2019;12:511–519. doi:10.2147/IDR.S192998
39. Paskova V, Medvecký M, Skalová A, et al. Characterization of NDM-encoding plasmids from *Enterobacteriaceae* recovered from Czech hospitals. *Front Microbiol*. 2018;9:1549. doi:10.3389/fmicb.2018.01549
40. Cuzon G, Bonnin RA, Nordmann P. First identification of novel NDM carbapenemase, NDM-7, in *Escherichia coli* in France. *PLoS One*. 2013;8:e61322. doi:10.1371/journal.pone.0061322
41. Devanga Ragupathi NK, Muthurilandi Sethuvel DP, Gajendiran R, et al. First Indian report of IncX3 plasmid carrying *bla*_{NDM-7} in *Escherichia coli* from bloodstream infection: potential for rapid dissemination. *New Microbes New Infect*. 2017;17:65–68. doi:10.1016/j.nmni.2017.01.012
42. Poirel L, Aires-de-Sousa M, Kudyba P, et al. Screening and characterization of multidrug-resistant gram-negative bacteria from a remote African area, São Tomé and Príncipe. *Antimicrob Agents Chemother*. 2018;62:e01021–18. doi:10.1128/AAC.01021-18
43. Sugawara Y, Akeda Y, Hagiya H, et al. Spreading patterns of NDM-producing *Enterobacteriaceae* in clinical and environmental settings in Yangon, Myanmar. *Antimicrob Agents Chemother*. 2019;63:e01924–18. doi:10.1128/AAC.01924-18

Supplementary materials

Table S1 Antibiotic susceptibility of clinical isolates carrying carbapenemases on IncX3 plasmids and their single IncX3 plasmid containing derivatives

Strain	Carbapenemase produced	Wild type/jugant/transformant	Ertapenem	Imipenem	Meropenem	Ceftazidime	Cefotaxime	Aztreonam	Ciprofloxacin	Gen-tamicin	Amikacin	Co-trimoxazole	Tetracycline	Colistin	Tigecycline	Fosfomycin
ABC220	KPC-2	WT	256	64	128	>128	>128	>128	64	256	16	>256	4	32	0.5	256
GM3163(pABC220-KPC-2)	KPC-2	TF	4	4	2	16	4	>128	≤0.125	1	1	≤0.5	≤0.5	≤0.5	≤0.125	16
ABC224	KPC-2	WT	256	64	128	>128	>128	>128	32	256	32	>256	4	32	0.5	128
ABC140	NDM-1	WT	2	32	8	128	32	4	8	2	4	≤0.5	16	>256	4	>512
DH5α(pABC140-NDM-1)	NDM-1	TF	0.5	2	≤0.25	>128	64	32	≤0.125	≤0.5	2	≤0.6	≤0.5	≤0.5	≤0.125	≤0.25
ABC40	NDM-1	WT	32	16	16	>128	>128	>128	>64	128	4	≤0.7	256	2	1	8
J53RAZ(pABC40-NDM-1)	NDM-1	TC	0.25	4	4	>128	64	32	≤0.125	≤0.5	≤0.5	≤0.8	≤0.5	≤0.5	≤0.125	0.5
ABC52	NDM-1	WT	64	32	64	>128	>128	>128	>64	>256	>256	>256	4	≤0.5	1	4
J53RAZ(pABC52-NDM-1)	NDM-1	TC	1	4	8	>128	64	64	≤0.125	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.125	1
ABC53	NDM-1	WT	64	64	64	>128	>128	>128	>64	>256	>256	>256	4	≤0.5	2	16
ABC54	NDM-1	WT	8	16	16	>128	128	>128	0.25	1	4	>256	128	≤0.5	0.25	0.5
DH5α(pABC54-NDM-1)	NDM-1	TF	0.25	4	4	>128	64	32	≤0.125	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.125	0.5
ABC80	NDM-1	WT	8	8	4	>128	128	>128	4	32	1	128	≤0.5	≤0.5	0.25	0.5
J53RAZ(pABC80-NDM-1)	NDM-1	TC	0.5	8	4	>128	64	32	≤0.125	1	≤0.5	≤0.5	≤0.5	≤0.5	≤0.125	0.5
BC680	NDM-1	WT	16	32	64	>128	>128	>128	>64	4	16	>256	2	≤0.5	2	4
BC700	NDM-1	WT	16	64	32	>128	>128	>128	>64	2	16	>256	2	≤0.5	2	4
J53RAZ(pBC700-NDM-1)	NDM-1	TC	2	4	4	>128	64	>128	≤0.125	1	≤0.5	≤0.5	≤0.5	≤0.5	≤0.125	0.5
BC-13-817	NDM-1	WT	16	32	32	>128	>128	>128	>64	4	16	>256	2	≤0.5	2	4
BC-13-836	NDM-1	WT	4	16	16	>128	>128	>128	≤0.125	2	4	≤0.5	128	≤0.5	≤0.125	0.5
DH5α(pBC836-NDM-1)	NDM-1	TF	0.25	2	≤0.25	>128	64	>128	≤0.125	≤0.5	1	≤0.5	≤0.5	≤0.5	≤0.125	≤0.25
ABC141	NDM-1+	WT	16	64	32	>128	>128	>128	>64	128	8	>256	>256	4	16	8
ABC155	OXA-48	WT	>256	>128	>128	>128	>128	>128	4	128	4	>256	>256	≤0.5	2	128
ABC137	NDM-1+	WT	16	64	32	>128	>128	>128	4	128	4	>256	>256	≤0.5	2	8
J53RAZ(pABC137-NDM-1)	NDM-1	TC	0.25	4	4	>128	64	32	≤0.125	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.125	0.5
ABC302	NDM-4	WT	128	64	128	>128	>128	>128	64	>256	>256	>256	8	≤0.5	2	16
DH5α(pABC302-NDM-4)	NDM-4	TF	0.25	2	≤0.25	>128	64	>128	≤0.125	≤0.5	≤0.5	≤0.5	≤0.5	≤0.5	≤0.125	≤0.25
ABC233	NDM-5	WT	32	16	32	>128	>128	>128	>64	>256	>256	128	1	≤0.5	≤0.125	0.5

(Continued)

Table S1 (Continued).

Strain	Carbapen- emase produced	Wild type/ transcon- jugant/ transfor- mant	Ertap- enem	Imi- mpe- nem	Mer- ope- nem	Ceft- azi- dime	Cefo- taxi- me	Aztr- eon- m	Cipro- flox- cin	Gen- tami- cin	Ami- kacin	Co-tri- moxa- zole	Tetr- acy- cline	Coli- stin	Tigec- ycline	Fosfo- mycin
DH5 α (pABC233-NDM-5)	NDM-5	TF	0.5	2	≤ 0.25	> 128	64	≤ 0.25	≤ 0.125	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.125	≤ 0.25
ABC268	NDM-5	WT	32	16	32	> 128	> 128	32	> 64	64	4	256	1	≤ 0.5	0.25	1
DH5 α (pABC268-NDM-5)	NDM-5	TF	0.25	2	≤ 0.25	> 128	64	≤ 0.25	≤ 0.125	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.125	≤ 0.25
ABC280	NDM-5	WT	32	16	16	> 128	> 128	> 128	> 64	64	8	> 256	1	≤ 0.5	0.25	1
J53RAZ(pABC280-NDM-5)	NDM-5	TC	0.5	4	8	> 128	128	≤ 0.25	≤ 0.125	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.125	1
ABC286	NDM-5	WT	64	8	32	> 128	> 128	> 128	> 64	32	4	256	1	≤ 0.5	0.25	1
ABC384	NDM-5	WT	64	128	32	> 128	> 128	> 128	> 64	64	8	256	> 256	≤ 0.5	≤ 0.125	0.5
J53RAZ(pABC384-NDM-5)	NDM-5	TC	0.25	4	4	> 128	64	≤ 0.25	≤ 0.125	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.125	1
ABC369	NDM-5+	WT	4	8	8	> 128	> 128	> 128	> 64	256	> 256	> 256	4	≤ 0.5	2	4
OXA-162																
DH5 α (pABC369-NDM-5)	NDM-5	TF	0.25	2	≤ 0.25	> 128	64	≤ 0.25	≤ 0.125	≤ 0.5	1	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.125	≤ 0.25
ABC133ETP	NDM-7	WT	128	128	128	> 128	> 128	64	> 64	64	8	≤ 0.5	> 256	≤ 0.5	0.25	0.5
DH5 α (pABC133-NDM)	NDM-7	TF	1	4	2	> 128	64	≤ 0.25	≤ 0.125	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.125	≤ 0.25
ABC218	NDM-7	WT	64	16	32	> 128	> 128	> 128	> 64	256	8	256	1	≤ 0.5	≤ 0.125	0.5
J53RAZ(pABC218-NDM)	NDM-7	TF	2	8	8	> 128	128	≤ 0.25	≤ 0.125	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.125	1
ABC239	OXA-181	WT	2	0.5	≤ 0.25	> 128	> 128	> 128	> 64	128	8	> 256	> 256	≤ 0.5	≤ 0.125	0.5
DH5 α (pABC239-OXA-181)	OXA-181	TF	0.5	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.125	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.125	≤ 0.25
ABC260	OXA-181	WT	128	64	32	2	2	0.5	16	1	1	> 256	4	16	2	> 512
DH5 α (pABC260-OXA-181)	OXA-181	TF	≤ 0.125	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.125	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.125	≤ 0.25
ABC264	OXA-181	WT	2	1	≤ 0.25	> 128	> 128	> 128	> 64	4	4	256	> 256	≤ 0.5	0.25	1
DH5 α (pABC264-OXA-181)	OXA-181	TF	≤ 0.125	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.125	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.125	≤ 0.25
ABC356	OXA-181	WT	2	1	0.5	> 128	> 128	> 128	> 64	128	8	> 256	> 256	≤ 0.5	0.25	0.5
DH5 α (pABC356-OXA-181)	OXA-181	TF	0.25	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.125	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.125	≤ 0.25
ABC381	OXA-181	WT	16	8	8	> 128	128	8	64	2	2	256	256	≤ 0.5	0.25	≤ 0.25
DH5 α (pABC381-OXA-181)	OXA-181	TF	≤ 0.125	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.125	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.125	≤ 0.25
BC-13-936	OXA-181	WT	0.25	2	0.5	≤ 0.25	≤ 0.25	≤ 0.25	1	2	2	> 256	1	≤ 0.5	0.25	16
BC-13-947	OXA-181	WT	≤ 0.125	2	0.5	16	2	64	1	2	2	> 256	1	8	0.25	16
DH5 α (pBC947-OXA-181)	OXA-181	TF	≤ 0.125	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.125	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.125	≤ 0.25
BC-13-970	OXA-181	WT	0.25	2	0.5	0.5	≤ 0.25	≤ 0.25	1	2	2	> 256	1	≤ 0.5	0.25	16
DH5 α	None	R	≤ 0.125	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.125	≤ 0.5	1	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.125	≤ 0.25
GM2163	None	R	≤ 0.125	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.125	≤ 0.5	4	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.125	16
J53Raz	None	R	≤ 0.125	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.25	≤ 0.125	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.125	0.5

Table S2 IncX3 plasmids (retrieved from GenBank in January 2019) from different geographical regions with unique backbone sequences and beta-lactamase genes carried

Resistance genes	Country	Name	GenBank Accession No
<i>bla_{KPC-2}</i>	Hong Kong	pKPC-NY79	JX104759
<i>bla_{NDM-17}</i>	China	pAD-19R	KX833071
<i>bla_{NDM-4}</i>	Myanmar	pM216_X3	AP018146
<i>bla_{NDM-4}</i>	Australia	pJEG027	KM400601
<i>bla_{NDM-4}</i>	Czech Republic	pEncl-922cz	MG252892
<i>bla_{NDM-5}</i>	Czech Republic	pEsco-5256cz	MG252891
<i>bla_{NDM-5}</i>	India	pNDM-MGR194	KF220657
<i>bla_{NDM-5}</i>	Hong Kong	pNDM-HK2998	MH234508
<i>bla_{NDM-5}</i>	Hong Kong	pNDM-HK2967	MH234509
<i>bla_{NDM-5}</i>	South Korea	pCREC-591_4	CP024825
<i>bla_{NDM-7}</i>	South Korea	pCREC-532_3	CP024833
<i>bla_{NDM-7}</i>	Oman	pOM26-NDM	KP776609
<i>bla_{NDM-7}</i>	Kuwait	pKW53T-NDM	KX214669
<i>bla_{NDM-7}</i>	Canada	pKpN01-NDM-7	CP012990
<i>bla_{NDM-7}</i>	Myanmar	pM110-X3	AP018141
<i>bla_{NDM-7}</i>	China	pEC50-NDM-7	KX470735
<i>bla_{OXA-181}</i>	Italy	pKP_BO_OXA-181	MG228426
<i>bla_{OXA-181}, qnrS1</i>	China	pOXA-181	KP400525
<i>bla_{OXA-181}, qnrS1</i>	Switzerland	pKS22	KT005457
<i>bla_{OXA-181}, qnrS1</i>	Germany	pOXA-181-IHIT35346	KX894452
<i>bla_{OXA-181}, qnrS1</i>	South Korea	pD6-OXA_I_I	MG702491
<i>bla_{OXA-181}, qnrS1</i>	Myanmar	pM206-OXA181	AP018831
<i>bla_{OXA-181}, qnrS1</i>	Czech Republic	pOXA181_29144	KX523903
<i>bla_{OXA-181}, qnrS1</i>	Lebanon	pSTIB_IncX3_OXA_181	MG570092
<i>bla_{OXA-181}, qnrS1</i>	Denmark	pAMA1167-OXA-181	CP024806
<i>bla_{SHV-11}</i>	Italy	pIncX-SHV	JN247852
<i>bla_{SHV-11}, bla_{KPC-3}</i>	Italy	p45-IncX3	KT362706
<i>bla_{SHV-12}</i>	Netherlands	pEC-393	KX618697
<i>bla_{SHV-12}</i>	Netherlands	pEC-125	KX618703
<i>bla_{SHV-12}, aac(6')-Ib</i>	USA	pKPN-819	CP008799
<i>bla_{SHV-12}, bla_{KPC-2}</i>	France	pKpS90	JX461340
<i>bla_{NDM-1}+ bla_{SHV-12}</i>	China	pNDM-HN380	JX104760
<i>bla_{NDM-1}+ bla_{SHV-12}</i>	Hong Kong	pNDM-HK3694	MH234505
<i>bla_{SHV-12}, bla_{TEM-1}, qnrS1</i>	Netherlands	pEC-NRS18	KX618696
None	USA	pUCLA OXA232-2	CP012563

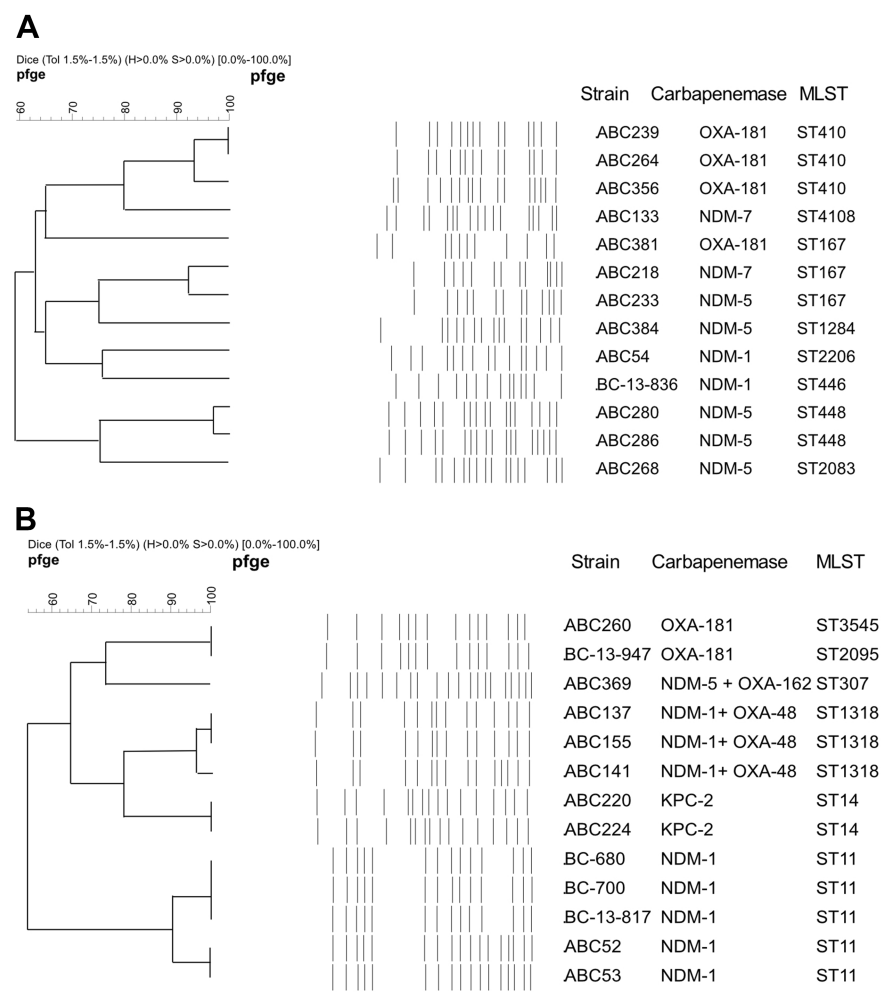


Figure S1 (A) Comparison of pulsed-field gel electrophoresis patterns of *Escherichia coli* isolates. **(B)** Comparison of pulsed-field gel electrophoresis patterns of *Klebsiella pneumoniae* isolates.

Infection and Drug Resistance

Dovepress

Publish your work in this journal

Infection and Drug Resistance is an international, peer-reviewed open-access journal that focuses on the optimal treatment of infection (bacterial, fungal and viral) and the development and institution of preventive strategies to minimize the development and spread of resistance. The journal is specifically concerned with the epidemiology of

antibiotic resistance and the mechanisms of resistance development and diffusion in both hospitals and the community. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/infection-and-drug-resistance-journal>