Infection and Drug Resistance

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

# 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 Darwish[1]
Akela Ghazawi[1]
Laura Villa[2]
Alessandra Carattoli[3]
Ágnes Sonnevend[1,4]

[1]Department of Medical Microbiology and Immunology, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates; [2]Department of Infectious Diseases, Istituto Superiore di Sanitá, Rome, Italy; [3]Department of Molecular Microbiology, University of Rome La Sapienza, Rome, Italy; [4]Department 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 $bla_{\text{NDM-1}}$, $bla_{\text{NDM-4}}$, $bla_{\text{NDM-5}}$, $bla_{\text{NDM-7}}$, $bla_{\text{OXA-181}}$ or $bla_{\text{KPC-2}}$ 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 $bla_{\text{OXA-181}}$ and $bla_{\text{NDM-5}}$ formed two distinct groups, whereas backbones of plasmids with $bla_{\text{NDM-1}}$, $bla_{\text{NDM-4}}$ and $bla_{\text{NDM-7}}$ 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

submit your manuscript | www.dovepress.com
DovePress
http://doi.org/10.2147/IDR.S210554

group (Inc) X defined as X3 type (IncX3)[6] have been reported worldwide in *Enterobacterales*, 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 Enterobacterales (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 in[23] 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* DH5α 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-trimoxasole, 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 $bla_{\text{NDM-1}}$ on IncX3 plasmid, and a further *Enterobacter cloacae* harbored IncX3-borne $bla_{\text{NDM-4}}$.

The 13 *E. coli* carried either $bla_{\text{NDM-1}}$, $bla_{\text{NDM-5}}$, $bla_{\text{NDM-7}}$ or $bla_{\text{OXA-181}}$ on IncX3 plasmids. They exhibited limited clonality; a triplet and two pairs of isolates formed PFGE clusters with ≥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 $bla_{\text{NDM-1}}$ exhibited ≥90% similar PFGE patterns, three NDM-1 and OXA-48 co-producing *K. pneumoniae* ST1318, with $bla_{\text{NDM-1}}$ 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 $bla_{\text{OXA-181}}$, and one had $bla_{\text{NDM-5}}$ on an IncX3 plasmid. This latter isolate, a *K. pneumoniae* ST307, co-produced NDM-5 and OXA-162, but $bla_{\text{OXA-162}}$ 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 |
|---|---|---|---|---|---|---|---|---|
| | 334 | 89 | 126 | 75 | 3 | 2 | 295 | 39 |
| IncX3 PCR positive | 32 (9.6%) | 18 (20%) | 6 (4.8%) | 4 (5.3%) | 0 | 2 (100%) | 30 (10.2%) | 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 | *E. coli* | NDM-7 | ST4108 | nonC | pABC133-NDM | 37070 | $bla_{NDM-7}$ | KX214671 | 24 |
| **ABC239** | 8/15/2013 | RH | Urine | *E. coli* | OXA-181 | ST410 | nonC | pABC239-OXA-181 | 51479 | $bla_{OXA-181}$+ *qnrS1* | MK412916 | This study |
| **ABC264** | 6/9/2014 | TH | Unknown | *E. coli* | OXA-181 | ST410 | nonC | pABC264-OXA-181 | 51479 | $bla_{OXA-181}$+ *qnrS1* | MK412917 | This study |
| **ABC356** | 8/8/2014 | MH | Urine | *E. coli* | OXA-181 | ST410 | Cm | pABC356-OXA-181 | 51479 | $bla_{OXA-181}$+ *qnrS1* | MK412918 | This study |
| **ABC381** | 11/4/2014 | AAH | Rectal swab | *E. coli* | OXA-181 | ST167 | nonC | pABC381-OXA-181 | 51479 | $bla_{OXA-181}$+ *qnrS1* | MK412919 | This study |
| ABC218 | 12/25/2012 | RH | Wound | *E. coli* | NDM-7 | ST167 | C | pABC218-NDM | 34403 | $bla_{NDM-7}$ | KX214670 | 24 |
| **ABC233** | 7/21/2013 | RH | Urine | *E. coli* | NDM-5 | ST167 | Cm | pABC233-NDM-5 | 46161 | $bla_{NDM-5}$ | MK372390 | This study |
| **ABC384** | 11/5/2014 | AAH | Urine | *E. coli* | NDM-5 | ST1284 | C | pABC384-NDM-5 | 46161 | $bla_{NDM-5}$ | MK372389 | This study |
| **ABC54** | 1/2/2011 | TH | Urine | *E. coli* | NDM-1 | ST2206 | C | pABC54-NDM-1 | 53023 | $bla_{NDM-1}$+ $bla_{SHV-12}$ | MK372382 | This study |
| **BC-13-836** | 9/24/2013 | TH | Blood | *E. coli* | NDM-1 | ST446 | C | pBC836-NDM-1 | 52565 | $bla_{NDM-1}$+ $bla_{SHV-11}$ | MK372387 | This study |
| **ABC280** | 7/15/2014 | TH | Urine | *E. coli* | NDM-5 | ST448 | C | pABC280-NDM5 | 35502 | $bla_{NDM-5}$ | MK372392 | This study |
| ABC286 | 8/15/2014 | TH | Blood | *E. coli* | NDM-5 | ST448 | NT | NT | NT | NT | NT | This study |
| **ABC268** | 6/11/2014 | AAH | Urine | *E. coli* | NDM-5 | ST2083 | Cm | pABC268-NDM-5 | 45232 | $bla_{NDM-5}$ | MK372391 | This study |
| **ABC40** | 10/27/2009 | TH | Wound | *E. cloacae* | NDM-1 | ST417 | Cm | pABC40-NDM-1 | 54035 | $bla_{NDM-1}$+ $bla_{SHV-12}$ | MK372380 | This study |
| **ABC302** | 2/26/2014 | MH | Urine | *E. cloacae* | NDM-4 | ST200 | C | ABC302-NDM-4 | 49402 | $bla_{NDM-4}$ | MK372388 | This study |
| **BC-13-947** | 7/11/2013 | TH | Blood | *K. pneumoniae* | OXA-181 | ST2095 | nonC | pBC947-OXA-181 | 51479 | $bla_{OXA-181}$+ *qnrS1* | MK412920 | This study |
| **ABC260** | 3/31/2014 | TH | Rectal swab | *K. pneumoniae* | OXA-181 | ST3545 | nonC | pABC260-OXA-181 | 51480 | $bla_{OXA-181}$+ *qnrS1* | MK412915 | This study |
| **ABC369** | 9/23/2014 | TH | Abdominal fluid | *K. pneumoniae* | NDM-5+ OXA-162 | ST307 | Cm | pABC369-NDM-5 | 45252 | $bla_{NDM-5}$ | MK372393 | This study |
| **ABC137** | 1/14/2013 | MH | Wound | *K. pneumoniae* | NDM-1+ OXA-48 | ST1318 | Cm | pABC137-NDM-1 | 53022 | $bla_{NDM-1}$+ $bla_{SHV-12}$ | MK372384 | This study |
| ABC141 | 4/20/2013 | MH | Unknown | *K. pneumoniae* | NDM-1+ OXA-48 | ST1318 | NT | NT | NT | NT | NT | This study |
| ABC155 | 6/5/2013 | SKMC | Blood | *K. pneumoniae* | NDM-1+ OXA-48 | ST1318 | NT | NT | NT | NT | NT | This study |
| ABC220 | 10/5/2012 | RH | Wound | *K. pneumoniae* | KPC-2 | ST14 | C# | pABC220-KPC-2 | 46900 | $bla_{KPC-2}$ | MK412914 | This study |
| ABC224 | 3/17/2013 | RH | Sputum | *K. pneumoniae* | KPC-2 | ST14 | C# | NT | NT | NT | NT | This study |
| **ABC52** | 9/19/2010 | TH | Sputum | *K. pneumoniae* | NDM-1 | ST11 | C | pABC52-NDM-1 | 52565 | $bla_{NDM-1}$+ $bla_{SHV-12}$ | MK372381 | This study |
| ABC53 | 9/19/2010 | TH | Sputum | *K. pneumoniae* | NDM-1 | ST11 | NT | NT | NT | NT | NT | This study |
| BC680 | 7/18/2012 | TH | Blood | *K. pneumoniae* | 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** | 7/24/2012 | TH | Blood | *K. pneumoniae* | NDM-1 | ST11 | C | pBC700-NDM-1 | 52565 | $bla_{NDM-1}$+ $bla_{SHV-11}$ | MK372386 | This study |
| BC-13-817 | 9/17/2013 | TH | Blood | *K. pneumoniae* | NDM-1 | ST11 | NT | NT | NT | NT | NT | This study |
| **ABC80** | 5/8/2011 | TH | Urine | *Citrobacter freundii* | NDM-1 | NT | Cm | pABC80-NDM-1 | 53023 | $bla_{NDM-1}$+ $bla_{SHV-12}$ | MK372383 | This study |
| **ABC140** | 3/25/2013 | MH | Perianal swab | *Morganella morganii* | NDM-1 | NA | nonC | pABC140-NDM-1 | 52591 | $bla_{NDM-1}$ | 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#, 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 UAE[24] (Table 2 and Figure 1).

In pABC220-KPC-2, the $bla_{KPC-2}$ gene was located on a Tn*4401b* 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 IS*26*.

The genetic load region of the eight $bla_{NDM-1}$ carrying plasmids was flanked by IS*26* and Tn*3*. The immediate genetic surrounding of the $bla_{NDM-1}$ between an IS*CR27* and a truncated IS*Aba125* was identical in all eight plasmids. The IS*26* bracketed composite transposon upstream of IS*CR27* either carried $bla_{SHV-11}$ (n=2), or $bla_{SHV-12}$ (n=5), or contained a truncated Tn*3* transposase (pABC140-NDM-1). The genetic surroundings of $bla_{NDM-4}$, $bla_{NDM-5}$ and $bla_{NDM-7}$ between IS*26* and IS*5* 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

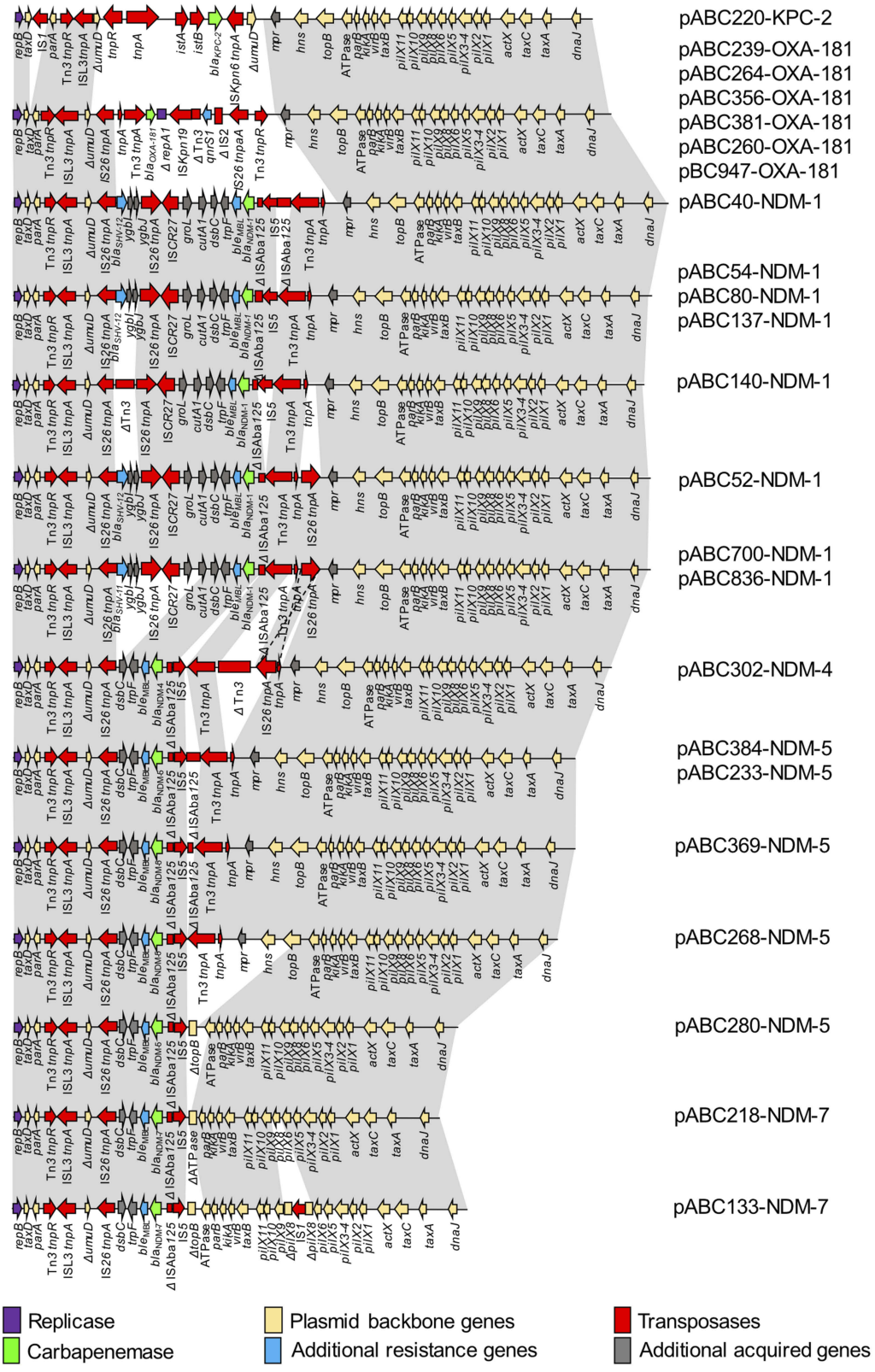


**Figure 1** Comparison of IncX3 plasmids from the United Arab Emirates carrying various carbapenemases.
**Notes:** Grey shades represent regions with ≥99% similarity.

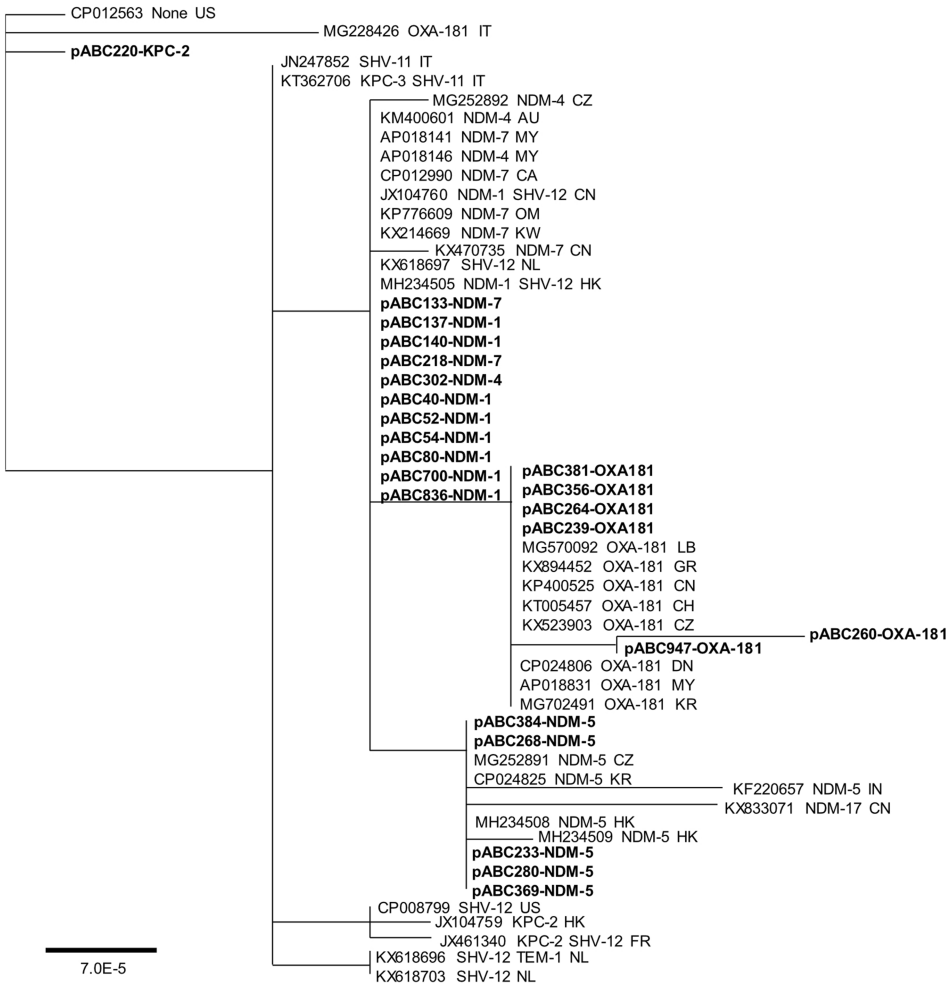


**Figure 2** Phylogenetic tree of backbone sequences of IncX3 plasmids from various geographical area.
**Notes:** The sequences were aligned using ClustalW, and the Neighbor-Joining tree was constructed using the Jukes-Cantor genetic distance model with 500 bootstrap replicates. All positions containing 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*$_{\text{SHV-12}}$ carrying plasmid from The Netherlands. The second one included *bla*$_{\text{NDM-5}}$ carrying plasmids, and the third clade clustered *bla*$_{\text{OXA-181}}$ carrying IncX3 plasmids originating from various parts of the world with a single outlier of *bla*$_{\text{OXA-181}}$ carrying IncX3 plasmid (MG228426) from Italy only. Conversely, plasmids harboring *bla*$_{\text{KPC}}$ 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*$_{\text{NDM-1}}$ 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 China[38] 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 France[40] 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 in[24]). 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

1. 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
2. van Duin D, Doi Y. The global epidemiology of carbapenemase-producing *Enterobacteriaceae*. *Virulence*. 2017;8:460–469. doi:10.1080/21505594.2016.1222343
3. 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
4. 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
5. 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
6. 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
7. 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
8. 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
9. 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
10. 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
11. 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
12. 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.
13. 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
14. 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
15. 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
16. 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
17. 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
18. 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
19. 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
20. 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
21. 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
22. 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
23. 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
24. 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
25. 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
26. 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
27. Cao V, Lambert T, Nhu DQ, et al. Distribution of extended-spectrum ß-lactamases in clinical isolates of *Enterobacteriaceae* in Vietnam *Antimicrob. Agents Chemother*. 2002;46:3739–3743. doi:10.1128/AAC.46.12.3739-3743.2002
28. *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.
29. 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. Gautom RK. Rapid pulsed-field gel electrophoresis protocol for typing of *Escherichia coli* O157: h7and 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 $bla_{\text{NDM-5}}$ -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, Medvecky M, Skalova 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, Muthuirulandi Sethuvel DP, Gajendiran R, et al. First Indian report of IncX3 plasmid carrying $bla_{\text{NDM-7}}$ 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/ transconjugant/ transformant | Ertapenem | Imipenem | Meropenem | Ceftazidime | Cefotaxime | Aztreonam | Ciprofloxacin | Gentamicin | 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 | ≤0.25 | ≤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 | ≤0.25 | ≤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 | ≤0.25 | ≤0.125 | ≤0.5 | 1 | ≤0.5 | ≤0.5 | ≤0.5 | ≤0.125 | ≤0.25 |
| ABC141 | NDM-1+ OXA-48 | WT | 16 | 64 | 32 | >128 | >128 | >128 | >64 | 128 | 8 | >256 | >256 | 4 | 16 | 8 |
| ABC155 | NDM-1+ OXA-48 | WT | >256 | >128 | >128 | >128 | >128 | >128 | 4 | 128 | 4 | >256 | >256 | ≤0.5 | 2 | 128 |
| ABC137 | NDM-1+ OXA-48 | 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 | ≤0.25 | ≤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 | Carbapenemase produced | Wild type/ transconjugant/ transformant | Ertapenem | Imipenem | Meropenem | Ceftazidime | Cefotaxime | Aztreonam | Ciprofloxacin | Gentamicin | Amikacin | Co-trimoxazole | Tetracycline | Colistin | Tigecycline | Fosfomycin |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DH5α(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 |
| DH5α(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+ OXA-162 | WT | 4 | 8 | 8 | >128 | >128 | >128 | >64 | 256 | >256 | >256 | 4 | ≤0.5 | 2 | 4 |
| DH5α(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 |
| DH5α(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 |
| DH5α(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 |
| DH5α(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 |
| DH5α(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 |
| DH5α(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 |
| DH5α(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 |
| DH5α(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 |
| DH5α | None | R | ≤0.125 | ≤0.25 | ≤0.25 | ≤0.25 | ≤0.25 | ≤0.25 | ≤0.125 | 1 | 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 | 2 | 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 | 1 | ≤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 |
|---|---|---|---|
| $bla_{KPC-2}$ | Hong Kong | pKPC-NY79 | JX104759 |
| $bla_{NDM-17}$ | China | pAD-19R | KX833071 |
| $bla_{NDM-4}$ | Myanmar | pM216_X3 | AP018146 |
| $bla_{NDM-4}$ | Australia | pJEG027 | KM400601 |
| $bla_{NDM-4}$ | Czech Republic | pEncl-922cz | MG252892 |
| $bla_{NDM-5}$ | Czech Republic | pEsco-5256cz | MG252891 |
| $bla_{NDM-5}$ | India | pNDM-MGR194 | KF220657 |
| $bla_{NDM-5}$ | Hong Kong | pNDM-HK2998 | MH234508 |
| $bla_{NDM-5}$ | Hong Kong | pNDM-HK2967 | MH234509 |
| $bla_{NDM-5}$ | South Korea | pCREC-591_4 | CP024825 |
| $bla_{NDM-7}$ | South Korea | pCREC-532_3 | CP024833 |
| $bla_{NDM-7}$ | Oman | pOM26-NDM | KP776609 |
| $bla_{NDM-7}$ | Kuwait | pKW53T-NDM | KX214669 |
| $bla_{NDM-7}$ | Canada | pKpN01-NDM-7 | CP012990 |
| $bla_{NDM-7}$ | Myanmar | pM110-X3 | AP018141 |
| $bla_{NDM-7}$ | China | pEC50-NDM-7 | KX470735 |
| $bla_{OXA-181}$ | Italy | pKP_BO_OXA-181 | MG228426 |
| $bla_{OXA-181}$, *qnr*S1 | China | pOXA-181 | KP400525 |
| $bla_{OXA-181}$, *qnr*S1 | Switzerland | pKS22 | KT005457 |
| $bla_{OXA-181}$, *qnr*S1 | Germany | pOXA-181-IHIT35346 | KX894452 |
| $bla_{OXA-181}$, *qnr*S1 | South Korea | pD6-OXA_1_1 | MG702491 |
| $bla_{OXA-181}$, *qnr*S1 | Myanmar | pM206-OXA181 | AP018831 |
| $bla_{OXA-181}$, *qnr*S1 | Czech Republic | pOXA181_29144 | KX523903 |
| $bla_{OXA-181}$, *qnr*S1 | Lebanon | pSTIB_IncX3_OXA_181 | MG570092 |
| $bla_{OXA-181}$, *qnr*S1 | Denmark | pAMA1167-OXA-181 | CP024806 |
| $bla_{SHV-11}$ | Italy | pIncX-SHV | JN247852 |
| $bla_{SHV-11}$, $bla_{KPC-3}$ | Italy | p45-IncX3 | KT362706 |
| $bla_{SHV-12}$ | Netherlands | pEC-393 | KX618697 |
| $bla_{SHV-12}$ | Netherlands | pEC-125 | KX618703 |
| $bla_{SHV-12}$, *aac(6')-Ib* | USA | pKPN-819 | CP008799 |
| $bla_{SHV-12}$, $bla_{KPC-2}$ | France | pKpS90 | JX461340 |
| $bla_{NDM-1}$+ $bla_{SHV-12}$ | China | pNDM-HN380 | JX104760 |
| $bla_{NDM-1}$+ $bla_{SHV-12}$ | Hong Kong | pNDM-HK3694 | MH234505 |
| $bla_{SHV-12}$, $bla_{TEM-1}$, *qnr*S1 | Netherlands | pEC-NRS18 | KX618696 |
| None | USA | pUCLAOXA232-2 | CP012563 |

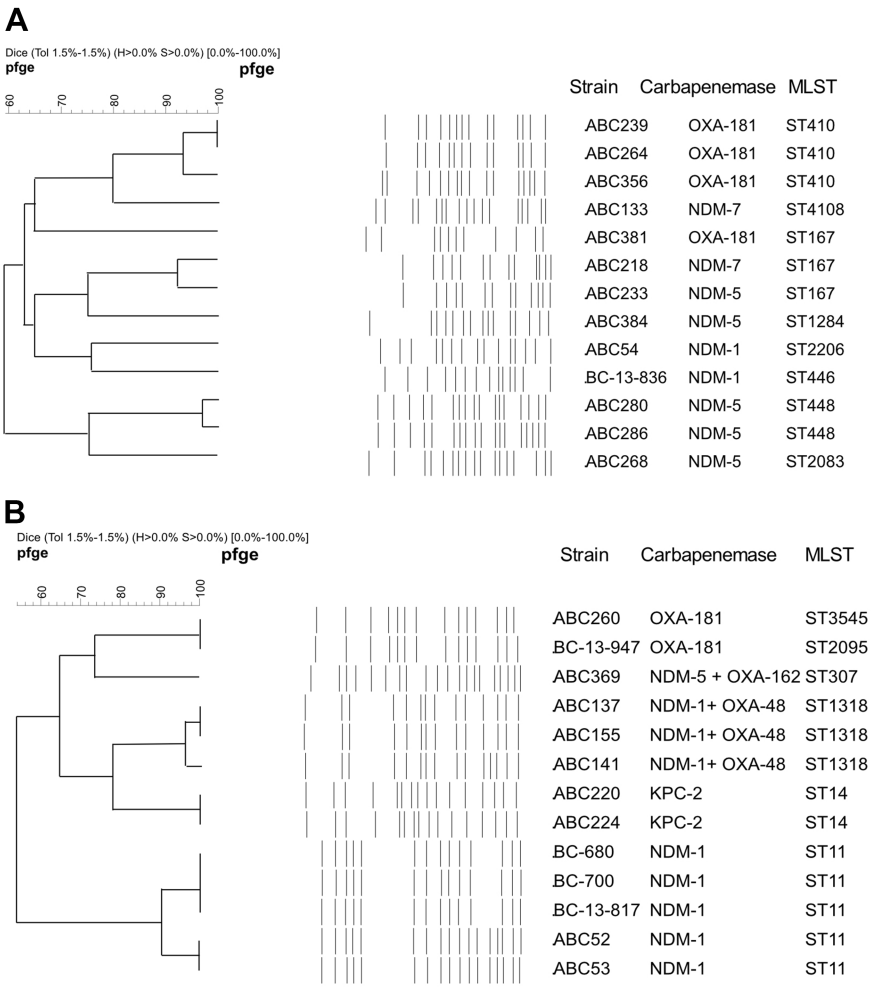


**Figure S1** (**A**) Comparison of pulsed-field gel electrophoresis patterns of *Escherichia coli* isolates. (**B**) Comparison of pulsed-field gel electrophoresis patterns of *Klebsiella pneumoniae* isolates.