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GENETIC SUPPORT OF CARBAPENEMASES IN DOUBLE CARBAPENEMASE PRODUCER *KLEBSIELLA PNEUMONIAE* ISOLATED IN THE ARABIAN PENINSULA

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Enterobacteriaceae co-producing NDM- and OXA-48-type carbapenemases were encountered in higher frequency in the United Arab Emirates (UAE) than in the neighboring countries in our earlier study. The aim of this investigation was to characterize the seven double carbapenemase producer *Klebsiella pneumoniae* found in the region to assess factors contributing to their emergence. Three *K. pneumoniae* ST14 isolated in the UAE harboring $bla_{\text{NDM-1}}$ on IncHI1b and $bla_{\text{OXA-232}}$ on IncColE plasmids were clonally related. Furthermore, two *K. pneumoniae* from the UAE, ABC106 and ABC137 belonged to ST307 and ST1318, respectively. ABC106 carried $bla_{\text{NDM-1}}$ on IncHI1b, and $bla_{\text{OXA-162}}$ on IncL/M plasmids, whereas ABC137 possessed $bla_{\text{NDM-1}}$ on IncX3 and $bla_{\text{OXA-48}}$ on IncL/M plasmids. The double carbapenemase-producing *K. pneumoniae* from Oman (OMABC109) and Saudi Arabia (SA54) belonged to ST11 and ST152, respectively. OMABC109 harbored $bla_{\text{NDM-1}}$ on an IncHI1b plasmid highly similar to the NDM-plasmid of ABC106 and carried a chromosomally coded $bla_{\text{OXA-181}}$ located on Tn*2013*. SA54 possessed a $bla_{\text{NDM-1}}$ on an IncFIb/FII plasmid and a $bla_{\text{OXA-48}}$ on an IncL/M plasmid. Based on these findings, clonal spread and horizontal transfer of carbapenemase genes located on transposons or self-transmissible plasmids contributed equally to the emergence of double carbapenemase-producing *Enterobacteriaceae* in the region.

Keywords: *Enterobacteriaceae*, carbapenem resistance, NDM- and OXA-48-type carbapenemases, Middle East

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Introduction

The emergence and rapid spread of carbapenem-resistant *Enterobacteriaceae* (CRE) is a major global concern [1]. In the majority of cases carbepenem resistance is due to the production of carbapenemases with varying levels of hydrolyzing activity against other beta-lactams. Carbapenemase enzymes belong either to Ambler class A, B, or D beta-lactamases. Their geographical distribution varies, but in general the most common ones are the KPC-, IMP-, VIM-, NDM-, and OXA-48-type enzymes. These enzymes often coded by genes located, as part of composite transposons, on conjugative plasmids that further facilitate their horizontal transfer [2].

It is likely that this mobile nature of carbapenemase genes has contributed to the emergence of *Enterobacteriaceae*, mostly *Klebsiella pneumoniae*, producing two or occasionally even three different carbapenemase genes. In Greece, where such isolates were first encountered, common combination of carbapenemases includes strains co-producing KPC-2 and various alleles of VIM [3, 4]. In other parts of the world, including the United States, Denmark, Turkey, Singapore, and India, strains expressing NDM-type carbapenemases together with OXA-48-like enzymes had emerged [5–9].

In a previous study investigating the molecular epidemiology of CRE in the Arabian Peninsula, we encountered five *Klebsiella pneumoniae* isolates co-producing NDM- and OXA-48-type carbapenemases in the United Arab Emirates (UAE) representing 8.9% of CRE isolates of this country. This was a considerable higher proportion than in other countries of the region [i.e., 1.9%, 0%, and 1.6% in the Kingdom of Saudi Arabia (KSA), Oman, and Kuwait, respectively] [10]. The aim of this study was to subject these five Emirati strains and the two isolates from the KSA and from Oman producing double carbapenemase to detailed molecular analysis to reveal whether the higher rate of such isolates in the UAE is due to a clonal expansion or, alternatively, to the emergence of unrelated strains.

Methods

Bacterial strains

The strains were encountered between April 2009 and 2013 during a previously published study [10]. The most important features of the isolates are summarized in Table I.

Table I. Clinical isolates characterized in the study

Strain	Country	Hospital/city	Date of isolatation	Sample	Species	Carbapenemases carried	Other resistance genes	PFGE type	MLST
ABC106	UAE	Tawam/ Al Ain	March 2012	Urine	*K. pneumoniae*	NDM-1 and OXA-162	$bla_{TEM-206}$, bla_{SHV-1}, $bla_{CTX-M-15}$, *qnrB*, *aac-6′-Ib-cr*, and *armA*	1	ST307
ABC120	UAE	Al Qasimi/ Sharjah	October 2011	Urine	*K. pneumoniae*	NDM-1 and OXA-232	bla_{TEM-1}, bla_{SHV-1}, $bla_{CTX-M-15}$, *qnrB*, *aac-6′-Ib-cr*, and *armA*	2	ST14
ABC127	UAE	Al Qasimi/ Sharjah	June 2012	Blood	*K. pneumoniae*	NDM-1 and OXA-232	bla_{SHV-1}, *qnrB*, *aac-6′-Ib-cr*, and *armA*	2	ST14
ABC128	UAE	Al Qasimi/ Sharjah	September 2012	Sputum	*K. pneumoniae*	NDM-1 and OXA-232	bla_{SHV-1}, *qnrB*, *aac-6′-Ib-cr*, and *armA*	2	ST14
ABC137	UAE	Mafraq/ Abu Dhabi	January 2013	Bedsore wound	*K. pneumoniae*	NDM-1 and OXA-48	bla_{TEM-1}, bla_{SHV-12}, $bla_{CTX-M-15}$, *qnrB*, and *aac-6′-Ib-cr*	3	ST1318
OM45	Oman	Royal/ Muscat	August 2011	Perianal swab	*K. pneumoniae*	NDM-1 and OXA-181	bla_{SHV-1}, $bla_{CTX-M-15}$, *qnrB*, *aac-6′-Ib-cr*, and *armA*	4	ST11
SA54	KSA	C/Riyadh	June 2012	Endotracheal aspirate	*K. pneumoniae*	NDM-1 and OXA-48	bla_{TEM-1}, bla_{SHV-1}, $bla_{CTX-M-15}$, *aac-6′-Ib-cr*, and *rmtC*	5	ST152

Note: UAE: United Arab Emirates; KSA: Kingdom of Saudi Arabia; PFGE: pulsed-field gel electrophoresis; MLST: multilocus sequence type.

Antibiotic susceptibility assays

Susceptibility to cefotaxime, ceftazidime, aztreonam, ertapenem, meropenem, imipenem, ciprofloxacin, gentamicin, amikacin, trimethoprime/sulfamethoxazole, tetracycline, chloramphenicol, and colistin was tested by broth microdilution, whereas tigecycline resistance was assessed by agar dilution [11]. For interpretation, the CLSI clinical breakpoints were used [11], with the exception of colistin and tigecycline interpreted by the EUCAST criteria (www.eucast.org).

Molecular characterization of the strains

The multilocus sequence type (MLST) of the isolates and the macrorestriction pattern of the *XbaI*-digested genomic DNA were established as described earlier [12, 13].

Resistance genes (bla_{TEM}, $bla_{CTX\text{-}M}$, bla_{SHV}, bla_{PER}, bla_{AmpC}, bla_{NDM}, $bla_{OXA\text{-}48\text{-}like}$, bla_{KPC}, bla_{VIM}, bla_{IMP}, *armA, rmtA, rmtB, rmtC, rmtD, qnrS, qepA*, and *aac6-1b-cr*) were detected by PCR, as previously described [10, 12, 13]. The specific alleles of bla_{TEM}, bla_{SHV}, and $bla_{CTX\text{-}M}$ beta-lactamase genes were determined by direct sequencing of the respective amplicons.

Characterizations of carbapenemase-bearing plasmids

Plasmids were routinely detected and sized by the alkaline lysis method of Kado and Liu [14] using the episomes in *E. coli* V517 [15] and *E. coli* 39R861 [16] as molecular mass standards. A Na-azide-resistant derivative of *E. coli* J53 ($J53_{RAZ}$) was used as recipient in conjugation. Competent cells of *E. coli* $J53_{RAZ}$ or of *E. coli* DH5α were used in transformation experiments. bla_{NDM} plasmids-carrying derivatives were selected on plates containing 8 mg/L ceftazidime and 150 mg/L Na-azide, whereas those carrying $bla_{OXA\text{-}48\text{-}like}$-bearing plasmids were selected on plates supplemented with 0.5 mg/L ertapenem, 200 mg/L dipicolinic acid, and 150 mg/L Na-azide. Transfer experiments were considered successful, if a single plasmid containing derivative was obtained. This was confirmed by PCR targeting the respective carbapenemase gene and by Southern blotting and hybridization of the plasmid electrophoresis gel. Attempts to detect extra-large plasmids were carried out by S1 nuclease digestions as described [13]. PCR-based replicon typing was done as described earlier [17, 18]. Southern blotting and hybridization of plasmids separated by regular gel electrophoresis or after S1 digestion was carried out as previously described [12].

Plasmid restriction fragment length polymorphism (RFLP), applying *BamH*I, *Sma*I, and *Xba*I restriction endonucleases, was used to compare plasmids of the same molecular mass and incompatibility type, carrying the same carbapenemase with identical flanking regions in clonally unrelated strains [12].

Characterization of the genetic environment of bla_{NDM} and $bla_{OXA\text{-}48\text{-}like}$ genes

The genetic scaffold of bla_{NDM} and bla_{OXA} was determined by PCR mapping, using crude plasmid extracts as a DNA template from the clinical isolates as described earlier [12]. This approach failed to map the bla_{NDM} surrounding of *K. pneumoniae* OMABC109 and ABC120, therefore, pOMABC109-NDM and pABC120-NDM were digested with *Hind*III restriction endonuclease, and the fragments were cloned into pUC19 and transformed into *E. coli* DH5α. The bla_{NDM}-bearing fragments cloned into pUC19 were selected on Triptic Soy Agar medium containing 8 mg/L ceftazidime. The bla_{NDM}-containing pUC19 plasmids were purified with Plasmid Mini Kit (Qiagen NV, Venlo, The Netherlands), and used as templates for sequencing the structures surrounding the bla_{NDM} [19].

Sequencing

PCR amplicons were purified with Wizard® SV Gel and PCR Clean-Up System (Pomega, USA). Plasmids were purified from single plasmid containing transconjugants or transformants using Plasmid Maxi Kit (Qiagen). Sequencing was performed with the Big Dye Cycle Terminator V.3.1 (Thermo Fisher Scientific, Waltham, MA, USA) on the 3130X Genetic Analyzer (Applied Biosystems) according to the manufacturer's instructions. The sequences obtained were analyzed using MEGA 4 and assembled with Clone Manager v9 software.

The assembled sequences were annotated using Sequin (http://www.ncbi.nlm.nih.gov/Sequin) and submitted to GenBank.

Results

Antibiotic susceptibility

All clinical isolates tested were multidrug resistant, i.e., resistant to all beta-lactam antibiotics tested with the exception of ABC127 and ABC128 remaining susceptible to aztreonam. The strains were also resistant to the majority of non-beta-lactam antibiotics tested. Nonetheless, all seven strains remained

susceptible to colistin, and none of them exhibited tigecycline resistance, although tigecycline MIC of ABC127, ABC128, and ABC137 exceeded the clinical breakpoint of 1 mg/L (Table II).

Molecular characteristics

The seven strains exhibited five distinct pulsed-field gel electrophoresis (PFGE) patterns, with one group clustering three of the strains also sharing the ST14 sequence type (Figure 1, Table I). The remaining four isolates were not related to this group and did not show similarity by PFGE or MLST (Table I). The specific MLST types and the antibiotic-resistance genes detected in each isolate are shown in Table I.

Localization of the carbapenemase genes

To reveal their possible plasmid localization, attempts were made to transfer the bla_{NDM} and $bla_{\text{OXA-48-like}}$ genes by conjugation into a Na-azide-resistant derivative of *E. coli* J53 ($J53_{\text{RAZ}}$). bla_{NDM}-containing transconjugants were obtained from all strains. However, in case of *K. pneumoniae* ABC137, the transconjugants obtained always carried multiple plasmid bands. Therefore, from one of these transconjugants, a crude plasmid extract was used to transform competent cells of $J53_{\text{RAZ}}$ resulting in single, bla_{NDM}-carrying plasmid containing derivatives.

Attempts to conjugally transfer of $bla_{\text{OXA-48-like}}$ carrying plasmid failed with all clinical isolates except for ABC106. However, we were successful to get single plasmid containing transformants carrying the respective $bla_{\text{OXA-48-like}}$ genes in *E. coli* DH5α using the crude plasmid extracts of ABC120, ABC127, ABC128, ABC137, and SA54.

Plasmid incompatibility types of carbapenemase-bearing plasmids were confirmed by PCR-based replicon typing, as well as by hybridization of the plasmid gels of the wild-type strains and single plasmid containing transconjugants or transformants. The size, incompatibility types, and genes co-transferring with the plasmids are shown in Table III. It should be noted that the approximately 110 kb NDM plasmid of SA54 harbored double replicase genes of incompatibility types FIb and FII. In this latter case, beyond PCR and hybridization, this incompatibility type was also confirmed by sequencing the respective region of the purified plasmid.

Transfer of the $bla_{\text{OXA-48-like}}$ gene from OMABC109 was unsuccessful either by conjugation or by transformation. Therefore, S1 nuclease digestion of

Table II. Antibiotic susceptibility of the double carbapenemase-producing wild type strains and their derivatives

Strain	Type of strain	Ceftazidime	Cefotaxime	Aztreonam	Ertapenem	Imipenem	Meropenem	Ciprofloxacin	Gentamicin	Amikacin	Co-trimoxazole	Tetracycline	Chloramphenicol	Colistin	Tigecycline
ABC106	W	>128	>128	>128	>64	32	32	64	>256	>256	>256/4864	256	>256	≤0.5	1
J(pABC106/13) NDM	TC	128	128	32	8	4	2	0.5	>256	>256	≤0.5/9.5	≤0.5	256	≤0.5	≤0.125
J(pABC106/4) OXA	TC	1	2	1	8	4	2	≤0.125	≤0.5	≤0.5	≤0.5/9.5	1	16	≤0.5	≤0.125
ABC120	W	>128	>128	>128	>64	128	128	>64	>256	>256	>256/4864	8	>256	≤0.5	1
J(pABC120/3) NDM	TC	128	64	≤0.25	8	4	2	0.5	>256	>256	≤0.5/9.5	≤0.5	8	≤0.5	≤0.125
D(pABC120 OXA T18)	TF	0.5	≤0.25	≤0.25	8	0.5	≤0.25	≤0.125	≤0.5	≤0.5	≤0.5/9.5	≤0.5	4	≤0.5	≤0.125
ABC127	W	>128	>128	2	>64	64	64	>64	256	>256	>256/4864	8	>256	>256	2
J(pABC127/2) NDM	TC	128	32	≤0.25	8	4	2	0.5	>256	>256	≤0.5/9.5	≤0.5	8	≤0.5	≤0.125
D(pABC127 OXA T11)	TF	0.5	≤0.25	≤0.25	8	0.5	≤0.25	≤0.125	≤0.5	≤0.5	≤0.5/9.5	≤0.5	2	≤0.5	≤0.125
ABC128	W	>128	>128	2	>64	128	128	>64	>256	>256	>256/4864	8	>256	≤0.5	2
J(pABC128/1) NDM	TC	128	64	≤0.25	4	4	2	0.5	>256	>256	≤0.5/9.5	≤0.5	8	≤0.5	≤0.125
D(pABC128 OXA T5)	TF	≤0.25	≤0.25	≤0.25	8	0.5	≤0.25	≤0.125	≤0.5	≤0.5	≤0.5/9.5	≤0.5	2	≤0.5	≤0.125
ABC137	W	>128	>128	>128	>64	64	64	4	128	4	>256/4864	>256	16	≤0.5	2
J(pABC137 NDM T1)	TF	>128	128	32	16	8	4	≤0.125	≤0.5	≤0.5	≤0.5/9.5	≤0.5	8	≤0.5	≤0.125

Table II. (cont.)

Strain	Type of strain	Ceftazidime	Cefotaxime	Aztreonam	Ertapenem	Imipenem	Meropenem	Ciprofloxacin	Gentamicin	Amikacin	Co-trimoxazole	Tetracycline	Chloramphenicol	Colistin	Tigecycline
D(pABC137 OXA T2)	TF	≤0.25	≤0.25	≤0.25	8	1	≤0.25	≤0.125	≤0.5	≤0.5	≤0.5/9.5	≤0.5	2	≤0.5	≤0.125
OMABC109	W	>128	>128	>128	>64	64	64	>64	>256	>256	>256/4864	2	>256	≤0.5	0.5
J(pOMABC109/16) NDM	TC	128	>128	16	4	4	1	0.25	>256	>256	≤0.5/9.5	≤0.5	256	≤0.5	≤0.125
SA54	W	>128	>128	>128	>64	64	64	64	>256	>256	>256/4864	1	8	≤0.5	0.25
J(pSA54/4) NDM	TC	>128	64	≤0.25	16	8	4	≤0.125	>256	>256	≤0.5/9.5	≤0.5	8	≤0.5	≤0.125
D(pSA54 OXA T8)	TF	≤0.25	1	≤0.25	16	2	≤0.25	≤0.125	≤0.5	32	≤0.5/9.5	≤0.5	2	≤0.5	≤0.125
J53RAZ	R	≤0.25	≤0.25	≤0.25	≤0.125	≤0.25	≤0.25	≤0.125	≤0.5	≤0.5	≤0.5/9.5	≤0.5	8	≤0.5	≤0.125
DH5α	R	≤0.25	≤0.25	≤0.25	≤0.125	≤0.25	≤0.25	≤0.125	≤0.5	≤0.5	≤0.5/9.5	≤0.5	2	≤0.5	≤0.125

Note: W: wild type; TF: transformant; TC: transconjugant; R: recipient.

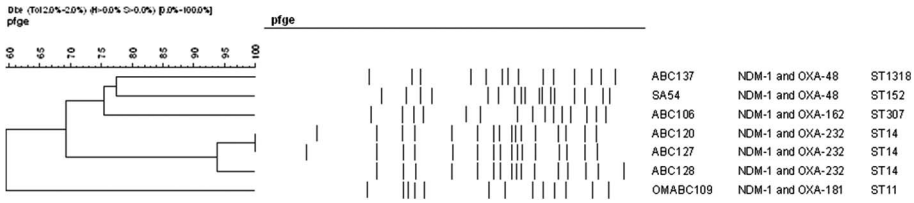

Figure 1. Comparison of pulsed-field gel electrophoresis (PFGE) patterns of double carbapenemase-producing *K. pneumoniae*

OMABC109 was carried out and the gel was hybridized using an OXA-181 probe, which localized the gene on the chromosome.

The genetic surrounding of the bla_{NDM} and $bla_{OXA\text{-}48\text{-}like}$ genes

To compare the genetic support of the respective carbapenemase genes, the sequence of the regions flanking them was also determined.

The regions downstream of the $bla_{\text{NDM-1}}$ genes were identical in all isolates containing a bleomycin-resistance gene ble_{MBL}. Immediate upstream of the $bla_{\text{NDM-1}}$ gene, all plasmids contained varying sizes of the 3′ end of the IS*Aba125*. Further upstream, the plasmids of the three clonally related isolates (ABC120, ABC127, and ABC128, respectively) were also identical containing an IS*Ec33* element. It was noteworthy that in the plasmids of ABC106 and OMABC109, while belonging to the same Inc type (HI1b) and exhibiting similar molecular mass (>160 kb), the region upstream of the carbapenemase gene contained a longer stretch of IS*Aba125* (254 bp instead of 101 bp) and an IS*3000* element. The respective regions of the NDM plasmids of ABC137 and SA54 were completely different. In the former one, an IS*5* element was identified, whereas the latter one contained a ribosomal methylase gene, *rmtC* upstream of a 97-bp-long 3′ end of IS*Aba125* transposase (Figure 2). The sequences surrounding of the $bla_{\text{NDM-1}}$ in ABC106, ABC120, ABC137, OMABC109, and SA54 isolates were deposited in the GenBank under accession numbers: MF774792, MF774793, MF774794, MF774795, and MF774796, respectively.

Although the class-D carbapenemases could not be mobilized by conjugation from six of the seven clinical isolates, mapping their genetic surrounding confirmed their location on mobile genetic elements. In pABC137-OXA, the $bla_{\text{OXA-48}}$ was located in a classical Tn*1999* transposon, in pSA54-OXA, the same allele was located in a Tn*1999* variant disrupted by IS*1R* both upstream and downstream of the carbapenemase gene. $bla_{\text{OXA-162}}$ was found in a Tn*1999.2* variant in the conjugative pABC106-OXA. In the Omani isolate OMABC109, the

Table III. Genetic support of carbapenemase genes

Strain	Location of the carbapenemase gene	Carbapenemase allele	Features of the carbapenemase gene-carrying plasmids			
			Transfer by	Size (kb)	Inc type	Genes co-transferring
ABC106	pABC106/13-NDM	NDM-1	Conjugation	>160	HI1b	$bla_{\text{CTX-M-15}}$, *qnrB*, *aac-6′-Ib-cr*, and *armA*
	pABC106/4-OXA	OXA-162	Conjugation	60	L/M	None
ABC120	pABC120/3-NDM	NDM-1	Conjugation	>160	HI1b	*qnrB*, *aac-6′-Ib-cr*, and *armA*
	pABC120-OXA-T18	OXA-232	Transformation	6	ColE	None
ABC127	pABC127/2-NDM	NDM-1	Conjugation	>160	HI1b	*qnrB*, *aac-6′-Ib-cr*, and *armA*
	pABC127-OXA-T11	OXA-232	Transformation	6	ColE	None
ABC128	pABC128/1-NDM	NDM-1	Conjugation	>160	HI1b	*qnrB*, *aac-6′-Ib-cr*, and *armA*
	pABC128-OXA-T5	OXA-232	Transformation	6	ColE	None
ABC137	pABC137-NDM-T1	NDM-1	Transformation	50	X3	$bla_{\text{SHV-12}}$
	pABC137-OXA-T2	OXA-48	Transformation	60	L/M	None
OMABC109	pOMABC109/16-NDM	NDM-1	Conjugation	>160	HI1b	$bla_{\text{CTX-M-15}}$, *qnrB*, *aac-6′-Ib-cr*, and *armA*
	Chromosome	OXA-181	None	–	–	–
SA54	pSA54/4-NDM	NDM-1	Conjugation	110	FIb/FII	*aac-6′-Ib-cr* and *rmtC*
	pSA54-OXA-T8	OXA-48	Transformation	60	L/M	None

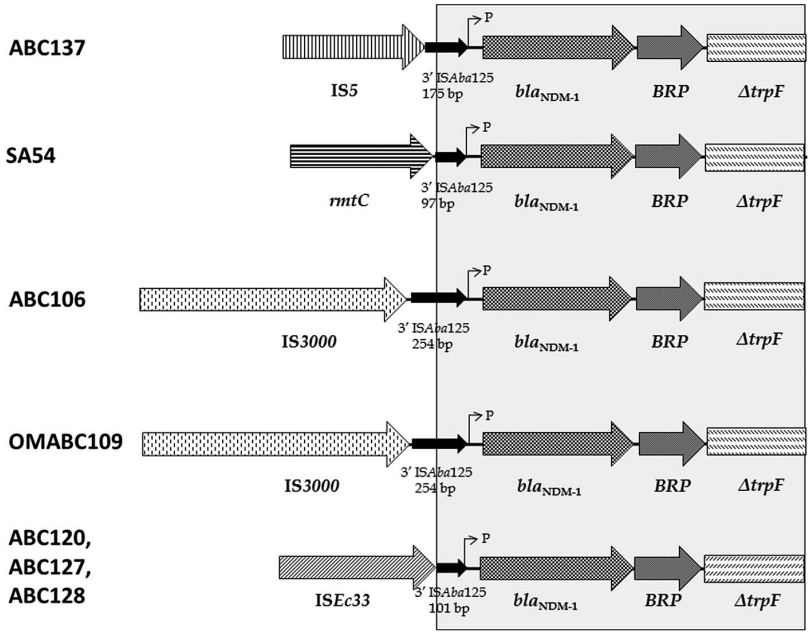

Figure 2. Genetic surrounding of $bla_{\text{NDM-1}}$ genes. The gray area indicates 100% identical regions

chromosomally located $bla_{\text{OXA-181}}$ was located in a Tn*2013* with IS*Ecp1* upstream of the carbapenemase gene (GenBank accession numbers: MF774788, MF774790, MF774787, and MF774789, respectively). In the clonally related isolates ABC120, ABC127, and ABC128, $bla_{\text{OXA-232}}$ was located on 6,141-bp-long ColE-type non-conjugative plasmid (MF774791) as part of a truncated Tn2013 transposon in which only the 206-bp 3′ end of the IS*Ecp1* was present upstream of the $bla_{\text{OXA-232}}$ gene (Figure 3). The complete sequence of this plasmid was almost identical to the first sequenced pOXA-232 (JX423831).

Plasmid RFLP

As OMABC109 *K. pneumoniae* ST11 and ABC106 *K. pneumoniae* ST307, i.e., clonally unrelated strains, carried the $bla_{\text{NDM-1}}$ on IncHI1b-type plasmids of similar size, and the genetic surrounding of $bla_{\text{NDM-1}}$ was identical in both, we analyzed the similarity of these plasmids by RFLP. Although the restriction patterns of the two plasmids were not completely identical, they showed a considerable level of similarity (Figure 4).

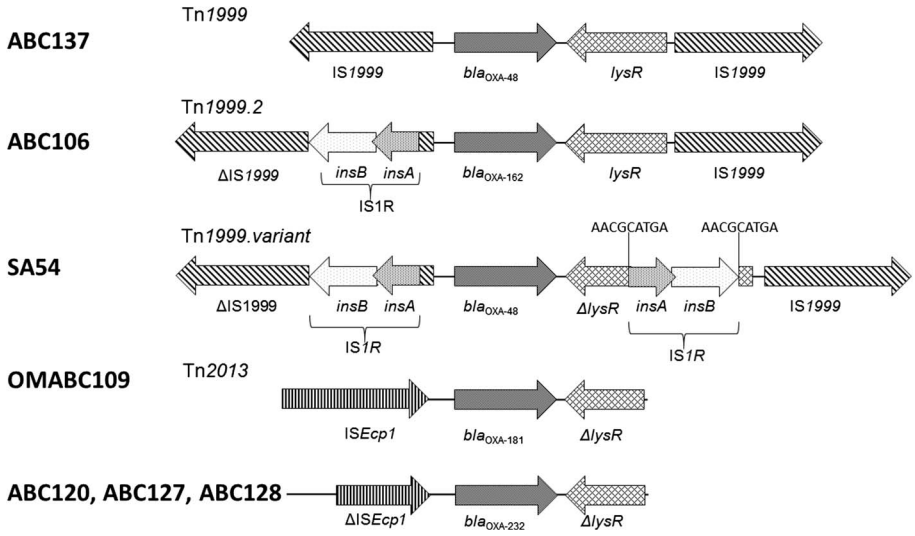

Figure 3. Genetic surrounding of $bla_{\text{OXA-48-type}}$ genes

Discussion

The possible advantages provided by the seemingly redundant expression of multiple carbapenemases by a strain are a matter of speculation. One may assume that the varying substrate profiles and hydrolytic efficacies of the different carbapenemases (e.g., the considerably reduced activity of some class D enzymes against cephalosporins) may explain the better survival chances of cells carrying multiple carbapenemase genes. Alternatively, or even simultaneously, as the different carbapenemase genes often reside on different genomic entities (i.e., on different plasmids or some on the chromosome, while others on plasmids) may secure that, particularly in the absence of selective pressure, a single genetic event will not result in a carbapenem-susceptible phenotype.

Whatever the reason is, the rate of double carbapenemase producers, particularly NDM and OXA carbapenemase co-producing *K. pneumoniae*, is emerging: beyond sporadic isolates detected in the USA, Singapore, Denmark, and India [5, 6, 8, 9], clusters of clonally spreading such *K. pneumoniae* have been described in Saudi Arabia [20]. Furthermore, a cluster of NDM-5 and OXA-181 co-producing pan-drug-resistant *K. pneumoniae* ST147 was also reported from the UAE [21], showing a further evolutionary step in the development of antibiotic resistance in this species. The strains investigated in this study were isolated earlier than the above pan-drug-resistant isolates [21], and showed no genetic relatedness

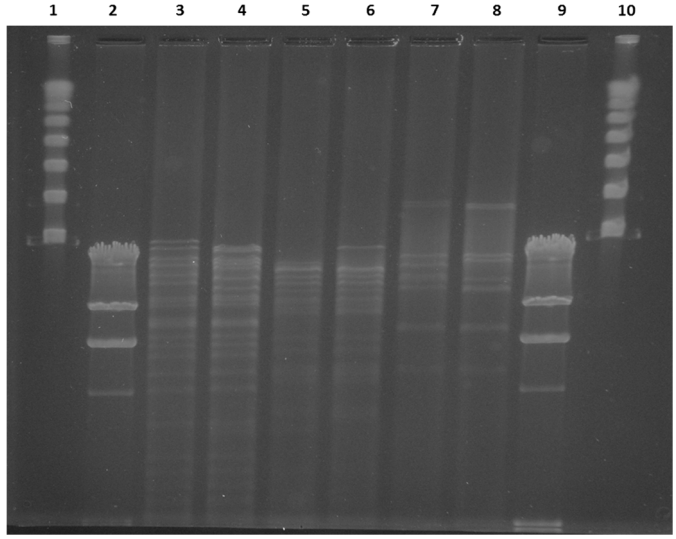

Figure 4. Plasmid restriction fragment length polymorphism of pABC106-NDM and pOMABC109-NDM. Lane 1 and 10 Lambda concatemer (NEBiolabs), lanes 2 and 9 Lambda phage DNA digested with *Hind*III restriction endonuclease, lane 3 pABC106-NDM digested with *BamH*I, lane 4 pOMABC109-NDM digested with *BamH*I, lane 5 pABC106-NDM digested with *Sma*I, lane 6 pOMABC109-NDM digested with *Sma*I, lane 7 pABC106-NDM digested with *Xba*I, and lane 8 pOMABC109-NDM digested with *Xba*I

to those strains. According to the results presented here, the higher proportion of NDM- and OXA-48-like enzyme co-producing clinical isolates in the UAE observed by us earlier [10] could only be partially explained by clonal expansion of *K. pneumoniae* ST14, since two of the five strains from the UAE had no genetic relatedness to these isolates or to each other. However, it is interesting to note that double carbapenemase-producing isolates of the UAE harbored NDM plasmids similar to ones described earlier from the region. *K. pneumoniae* ABC137 carried $bla_{\text{NDM-1}}$ on an IncX3-type plasmid shown earlier to contribute to the spread of this carbapenemase in the UAE [12]. The other four isolates carried $bla_{\text{NDM-1}}$ on IncHI1b-type plasmids, which was encountered earlier in the UAE and also in Oman [12, 22]. Furthermore, the highly similar pABC106-NDM of ABC106 *K. pneumoniae* ST307 from the UAE and pOMABC109-NDM of OMABC109 *K. pneumoniae* ST11 from Oman showed similarities to pNDM-MAR of a *K. pneumoniae* ST15 from Morocco, having identical incompatibility type and regions flanking $bla_{\text{NDM-1}}$, and also carrying $bla_{\text{CTX-M-15}}$ and *qnrB1* genes [23]. The same incompatibility-type plasmid, but with different insertion element upstream of $bla_{\text{NDM-1}}$, was present in the three clonally related *K. pneumoniae*

ST14. All these findings suggest the ability of this conjugative plasmid to establish itself in global multidrug-resistant clones of *K. pneumoniae.*

Contrary to the NDM-plasmids, the majority of OXA-plasmids encountered were not self-transmissible, although, with the exception of the $bla_{\text{OXA-232}}$ genes, all were located on mobile genetic elements known to be associated with the spread of these enzymes [24]. Nevertheless, $bla_{\text{OXA-232}}$ spread here due to the clonal expansion of *K. pneumoniae* ST14. This observation is in line with previous findings, as this particular carbapanemase was first described in *K. pneumoniae* ST14, and one of the first double carbapenemase-producing isolates reported was also a *K. pneumoniae* ST14 co-producing NDM-1 and OXA-232 [5, 25].

Characterization of these double carbapenemase-producing *K. pneumoniae* encountered in the early phase of spread of CRE in the UAE illustrates the complexity of the factors contributing to emergence of multidrug-resistant organisms. Unlike in a classical outbreak situation largely due to clonal spread, double carbapenemase-producing *Enterobaceriaceae* seem to have evolved due to horizontal transfer of mobile genetic elements encountered earlier either in the region or globally [10, 12, 24]. This latter way of transmission of antibiotic resistance threatens the efficiency of infection prevention, originally designed to control transfer of strains, i.e., their clonal spread and leaves antimicrobial stewardship exercised across the whole spectrum of human and veterinary medicine the only possible way to lessen the frequency of such horizontal gene transfer events.

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Conflict of Interest

No competing financial interests exist.

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