



Multihospital Occurrence of Pan-Resistant *Klebsiella pneumoniae* Sequence Type 147 with an ISEcp1-Directed *bla*_{OXA-181} Insertion in the *mgrB* Gene in the United Arab Emirates

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ABSTRACT The emergence of pan-resistant *Klebsiella pneumoniae* strains is an increasing concern. In the present study, we describe a cluster of 9 pan-resistant *K. pneumoniae* sequence type 147 (ST147) isolates encountered in 4 patients over nearly 1 year in 3 hospitals of the United Arab Emirates (UAE). The isolates exhibited highly similar genotypes. All produced chromosomally encoded OXA-181, and the majority also produced the NDM-5 carbapenemase. As with the previously described single isolate from the UAE, MS6671, the *mgrB* was disrupted by a functional, ISEcp1-driven *bla*_{OXA-181} insertion causing resistance to carbapenems. The mutation was successfully complemented with an intact *mgrB* gene, indicating that it was responsible for colistin resistance. *bla*_{NDM-5} was located within a resistance island of an approximately 100-kb IncFII plasmid carrying *ermB*, *mph(A)*, *bla*_{TEM-1B1}, *rmtB*, *bla*_{NDM-5}, *sul1*, *aadA2*, and *dfpA12* resistance genes. Sequencing this plasmid (pABC143-NDM) revealed that its backbone was nearly identical to that of plasmid pMS6671E from which several resistance genes, including *bla*_{NDM-5}, had been deleted. More extensive similarities of the backbone and the resistance island were found between pABC143C-NDM and the *bla*_{NDM-5}-carrying IncFII plasmids of two *K. pneumoniae* ST147 isolates from South Korea, one of which was colistin resistant, and both also produced OXA-181. Notably, one of these strains was isolated from a patient transferred from the UAE. Our data show that this pan-resistant clone has an alarming capacity to maintain itself over an extended period of time and is even likely to be transmitted internationally.

KEYWORDS pan-resistance, *Klebsiella pneumoniae*, carbapenems, colistin

Klebsiella pneumoniae has long been recognized to cause severe community or hospital-acquired infections (1, 2) and also to gain resistance relatively easily to multiple antibiotics (2, 3). Certain clones (e.g., clonal complex [CC] 258 or sequence type [ST] 147) are particularly important in disseminating multidrug resistance globally, including to carbapenems. Resistance to these later drugs is commonly due to the

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production of various carbapenemases, such as KPC, NDM, VIM, and various alleles of OXA-type enzymes (3–6).

Management of infections caused by carbapenem-resistant *K. pneumoniae* often includes polymyxins, typically colistin (7). Over the last decades, strains with decreased susceptibility to polymyxins have started to emerge (8). Colistin resistance is commonly due to the altered physicochemical features of the bacterial cell wall that limit the capacity of the cationic polymyxin molecules to bind to it. This can be the result of mutations in several chromosomal genes, of which the most common is *mgrB* (9). Recently, genes responsible for colistin resistance located on mobile genetic elements have also been described, further increasing the chance to spread resistance to polymyxins (10).

Although the lack of susceptibility to polymyxins in carbapenem-resistant strains does not mean resistance to all antibiotics, *per se*, it carries an increased risk of the emergence of pan-drug-resistant (PDR) clones. Indeed, several PDR *K. pneumoniae* strains, some even causing outbreaks, have already been described (11, 12).

In the present study, we report on a cluster of NDM-5- and OXA-181-producing, pan-resistant *K. pneumoniae* ST147 strains encountered in two emirates of the United Arab Emirates (UAE) over a period of 1 year, with possible connections to strains subsequently isolated in South Korea. Furthermore, we also provide evidence that the molecular basis of colistin resistance in the cluster was due to the insertion of a functional *bla*_{OXA-181} gene into *mgrB*.

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RESULTS

Collection of strains. In May 2013, four *K. pneumoniae* isolates (ABC143A to -D) were received for molecular typing. They were recovered from various samples from the same patient in an Abu Dhabi hospital (AD hospital) and were resistant to all antibiotics tested by the hospital laboratory. The patient, who had peritonitis from a leaking anastomosis after an operation for obstruction and perforation of the small intestine, had been transferred from an Umm Al Quwain hospital (UAQ hospital A). A week later another pan-resistant isolate (ABC146) was obtained from the same Abu Dhabi hospital, this one recovered from the sputum of a 75-year-old male patient treated during the same period of time, in the same ward. In late May, two more isolates were recovered from the first patient's blood and peritoneal aspirate (ABC143E and ABC143F).

In August, a PDR strain from UAQ hospital A, i.e., the same hospital from which the first patient had been transferred to Abu Dhabi, was sent to our laboratory. Strain ABC164 was isolated from a sample taken from the mastectomy wound of a 75-year-old, septic, ventilated female patient with advanced breast cancer. According to the hospital records, all three patients succumbed due to sepsis.

We compared these isolates to another PDR *K. pneumoniae* strain (MS6671) described earlier (12) from a different hospital at Um al Quwain, i.e., UAQ hospital B; this strain was isolated 7 months after the first isolate in UAQ hospital A had been encountered. The characteristics of the isolates are shown in Table 1.

Antibiotic susceptibility. All strains exhibited nonsusceptibility to all antibiotics tested, including colistin, fosfomycin, and tigecycline. The MICs of benzalkonium and irgasan for the strains were similar to the MICs for the *Escherichia coli* K-12 laboratory strain; however, the chlorhexidine MICs for the clinical isolates were considerably higher (Table 2).

Molecular characterization. The isolates from the AD hospital and from UAQ hospital A clustered by both repetitive elements PCR (rep-PCR; DiversiLab, bioMérieux) and pulsed-field gel electrophoresis (PFGE). Although *K. pneumoniae* MS6671 exhibited a considerably different pattern by rep-PCR, its PFGE pattern was still >80% similar to the PFGE patterns of the *K. pneumoniae* isolated from the other 3 patients (Fig. 1). All

TABLE 1 Characteristics of the pan-drug-resistant *K. pneumoniae* isolates

Isolate	Hospital of isolation	Specimen	Date of isolation	Virulence factor genes	Resistance genes ^a
ABC143A	Abu Dhabi hospital	Body fluid	12 May 2013	<i>fimH, traT, ureA, uge, wabG, mrkD, fyuA</i>	<i>bla</i> _{NDM-5} , <i>bla</i> _{TEM-1B} , <i>rmtB, rmtF, bla</i> _{SHV-36} , <i>bla</i> _{CTX-M-15} , <i>bla</i> _{OXA-181} , <i>aac6'-1b-cr, qacΔE, cepA</i>
ABC143B	Abu Dhabi hospital	Sputum	11 May 2013	<i>fimH, traT, ureA, uge, wabG, mrkD, fyuA</i>	<i>bla</i> _{NDM-5} , <i>bla</i> _{TEM-1B} , <i>rmtB, rmtF, bla</i> _{SHV-36} , <i>bla</i> _{CTX-M-15} , <i>bla</i> _{OXA-181} , <i>aac6'-1b-cr, qacΔE, cepA</i>
ABC143C	Abu Dhabi hospital	Blood	12 May 2013	<i>fimH, traT, ureA, uge, wabG, mrkD, fyuA</i>	<i>bla</i>_{NDM-5}, <i>bla</i>_{TEM-1B}, <i>rmtB, rmtF, bla</i>_{SHV-36}, <i>bla</i>_{CTX-M-15}, <i>bla</i>_{OXA-181}, <i>aac6'-1b-cr, qacΔE, cepA</i>
ABC143D	Abu Dhabi hospital	Perianal swab	11 May 2013	<i>fimH, traT, ureA, uge, wabG, mrkD, fyuA</i>	<i>bla</i> _{NDM-5} , <i>bla</i> _{TEM-1B} , <i>rmtB, rmtF, bla</i> _{SHV-36} , <i>bla</i> _{CTX-M-15} , <i>bla</i> _{OXA-181} , <i>aac6'-1b-cr, qacΔE, cepA</i>
ABC143E	Abu Dhabi hospital	Blood	29 May 2013	<i>fimH, traT, ureA, uge, wabG, mrkD, fyuA</i>	<i>bla</i> _{NDM-5} , <i>bla</i> _{TEM-1B} , <i>bla</i> _{SHV-36} , <i>bla</i> _{CTX-M-15} , <i>bla</i> _{OXA-181} , <i>rmtF, aac6'-1b-cr, qacΔE, cepA</i>
ABC143F	Abu Dhabi hospital	Body fluid	29 May 2013	<i>fimH, traT, ureA, uge, wabG, mrkD, fyuA</i>	<i>bla</i> _{TEM-1B} , <i>bla</i> _{SHV-36} , <i>bla</i> _{CTX-M-15} , <i>bla</i> _{OXA-181} , <i>rmtF, aac6'-1b-cr, cepA</i>
ABC146	Abu Dhabi hospital	Sputum	21 May 2013	<i>fimH, traT, ureA, uge, wabG, mrkD, fyuA</i>	<i>bla</i> _{NDM-5} , <i>bla</i> _{TEM-1B} , <i>rmtB, rmtF, bla</i> _{SHV-36} , <i>bla</i> _{CTX-M-15} , <i>bla</i> _{OXA-181} , <i>aac6'-1b-cr, qacΔE, cepA</i>
ABC164	Um al Quwain hospital A	Pus	18 August 2013	<i>fimH, traT, ureA, uge, wabG, mrkD, fyuA</i>	<i>bla</i> _{NDM-5} , <i>bla</i> _{TEM-1B} , <i>rmtB, rmtF, bla</i> _{CTX-M-15} , <i>bla</i> _{OXA-181} , <i>aac6'-1b-cr, qacΔE, cepA</i>
MS6671 ^b	Um al Quwain hospital B	Urine	26 March 2014	<i>fimH, traT, ureA, uge, wabG, mrkD, fyuA</i>	<i>bla</i> _{SHV-36} , <i>bla</i> _{CTX-M-15} , <i>bla</i> _{OXA-181} , <i>aac6'-1b-cr, cepA, rmtF</i>

^aAs detected by PCR amplification and sequencing. The genes in bold were also detected in the pABC143C-NDM complete sequence by next-generation sequencing. ^bSee reference 12.

isolates tested belonged to ST147. The genotypes of the strains were also highly similar (Table 1).

All strains carried both *bla*_{OXA-181} and *bla*_{NDM-5} carbapenemase genes, with the exception of MS6671 and one isolate (ABC143F) from the first patient, which were positive for *bla*_{OXA-181} only. By hybridization, the *bla*_{NDM-5} gene could be located on an approximately 100-kb plasmid, but the OXA-181 probe did not hybridize with any of the plasmids (data not shown). On the other hand, hybridization of the XbaI-digested and PFGE-separated full genome of the strains with an OXA-181 probe revealed 3 bands of the same sizes as those seen in MS6671, reported to carry three chromosomal copies of *bla*_{OXA-181} (12). One isolate from the first patient (ABC143F) also exhibited an additional, fourth, smaller fragment hybridizing with the OXA probe (Fig. 2).

PCR of the *mgrB* gene of all isolates resulted in an approximately 3,000-bp amplicon, suggesting a large insertion into the gene. Sequencing of the amplicons from all nine isolates revealed an ISEcp1-associated *bla*_{OXA-181} gene inserted into the *mgrB* (Fig. 3). In order to prove the functionality of the inserted carbapenemase gene, the entire *mgrB::bla*_{OXA-181} amplicons from ABC143C and MS6671 were cloned into pUC19, resulting in plasmids pCoxABC143C and pCoxMS6671. These plasmids were transformed into *E. coli* DH5α. The transformants exhibited ertapenem and meropenem resistance

TABLE 2 Antibiotic and disinfectant susceptibility of pan-drug-resistant *K. pneumoniae* isolates and the transconjugant

Strain	MIC (mg/liter) ^a															
	IMI	MEM	ERT	CAZ	CTX	AZT	CIP	GM	AM	SXT	TIG	FOS	COL	BAC	IRG	CHX
ABC143A	>128	>128	>64	>128	>128	128	>64	>256	>256	>256/4864	8	128	16	128	1	32
ABC143B	>128	>128	>64	>128	>128	128	64	>256	>256	>256/4864	2	128	8	64	<0.5	32
ABC143C	>128	>128	>64	>128	>128	128	64	>256	>256	>256/4864	2	128	8	64	<0.5	32
ABC143D	>128	>128	>64	>128	>128	128	>64	>256	>256	>256/4864	4	128	8	64	1	32
ABC143E	>128	>128	>64	>128	>128	>128	64	>256	>256	>256/4864	4	64	>256	64	<0.5	32
ABC143F	16	32	>64	32	>128	128	>64	>256	>256	4/76	8	256	4	64	<0.5	16
ABC146	16	128	>64	>128	>128	>128	64	>256	>256	>256/4864	2	64	8	64	<0.5	32
ABC164	128	>128	>64	>128	>128	>128	>64	>256	>256	>256/4864	2	64	32	64	<0.5	32
MS6671	16	>128	>64	64	>128	>128	>64	>256	>256	8/152	2	64	32	64	1	32
J53RAZ(pABC143C/1)	4	16	8	>128	64	<0.25	<0.125	256	256	<0.5/9.5	<0.125	1	<0.5	128	<0.5	2
J53RAZ	<0.25	<0.25	<0.125	<0.25	<0.25	<0.25	<0.125	<0.5	<0.5	<0.5/9.5	<0.125	1	<0.5	128	<0.5	2

^aIMI, imipenem; MEM, meropenem; ETP, ertapenem; CAZ, ceftazidime; CTX, cefotaxime; AZT, aztreonam; CIP, ciprofloxacin; GM, gentamicin; AM, amikacin; SXT, trimethoprim-sulfamethoxazole; TIG, tigecycline; COL, colistin; FOS, fosfomycin; BAC, benzalkonium; IRG, irgasan; CHX, chlorhexidine.

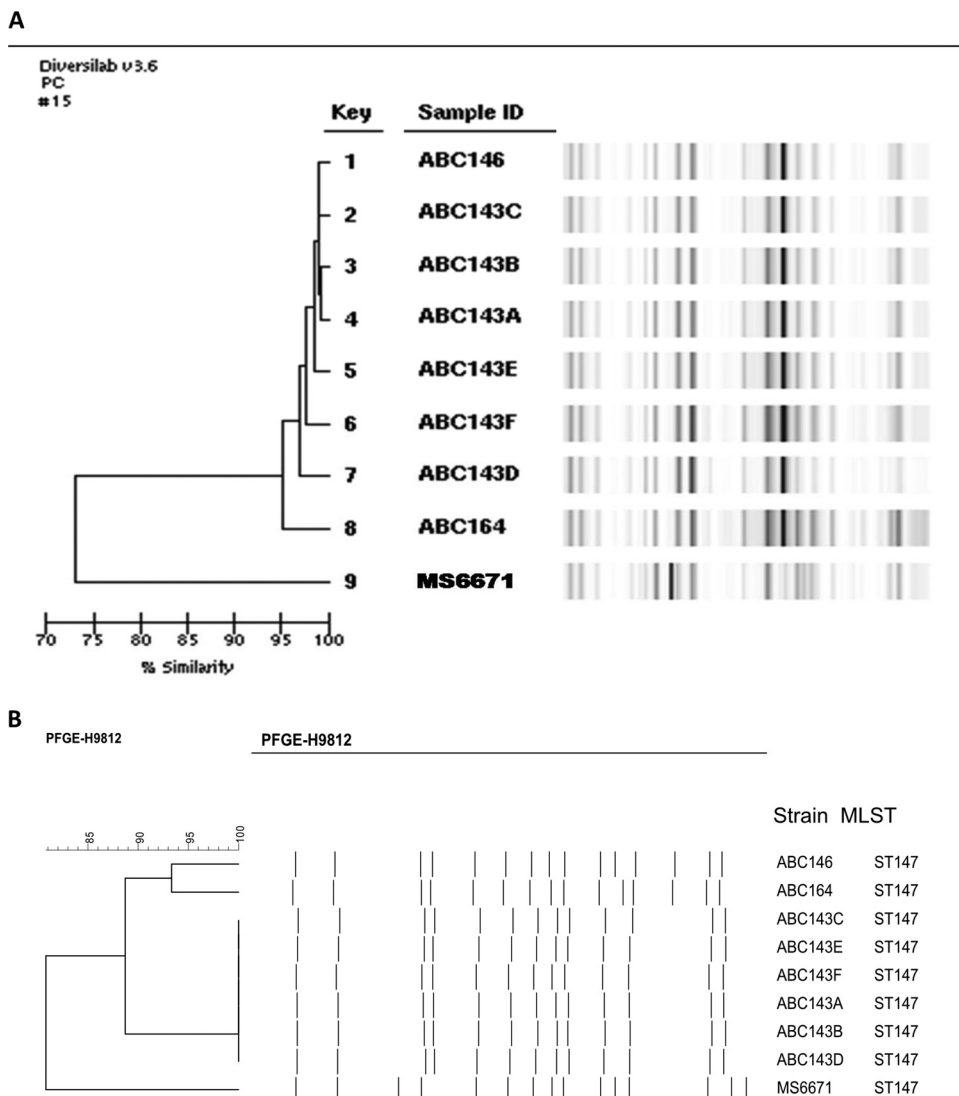


FIG 1 Comparative typing of members of the cluster. (A) rep-PCR (DiversiLab); (B) PFGE.

while maintaining susceptibility to ceftazidime, cefotaxime, and aztreonam, respectively, suggestive of a functional OXA-181 carbapenemase (Table 3).

The effect of the *mgrB::bla_{OXA-181}* insertion on the strains' susceptibility to colistin was successfully reversed by introducing pComgrB, i.e., the pDG106 vector carrying an intact *mgrB* gene. Both ABC143C(pComgrB) and MS6671(pComgrB) regained their susceptibility to colistin, while the transformants carrying the vector only did not (Table 4).

Plasmid pABC143C-NDM carrying the *bla_{NDM-5}* gene. We obtained the complete sequence of the plasmid present in most strains of the cluster carrying the *bla_{NDM-5}* gene from *K. pneumoniae* ABC143C. pABC143C-NDM (GenBank accession no. [KY130431](#)) is an IncFII incompatibility type plasmid of 96,549 bp with a GC content of 52.8%. It has 119 coding sequences, of which eight encode antibiotic resistance: *ermB*, *mph(A)*, *bla_{TEM-1B'}*, *rmtB*, *bla_{NDM-5'}*, *sul1*, *aadA2*, and *dfrA12* (Fig. 4). These genes are located in a resistance island inserted into the 66,915-bp-long plasmid backbone, which is highly similar to that of plasmid pMS6671E of MS6671 (GenBank accession no. [LN824138](#)), i.e., the *bla_{NDM}*-negative strain recovered in UAQ hospital B (12). Even more similarities were revealed with two other IncFII-type plasmids, both carrying *bla_{NDM-5'}*, i.e., pCC1410-1 and pCC1409-1 (GenBank accession no. [KT725788](#) and [KT725789](#), respectively) from strains isolated in South Korea (5, 13). The pMS6671E plasmid carried

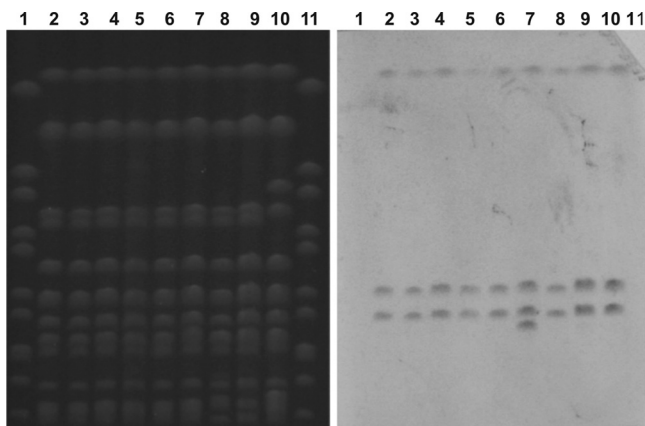


FIG 2 Hybridization of the genomic DNA fragments of strains with an OXA-181 probe. Samples: 1 and 11, *Salmonella enterica* serovar Braenderup strain H9812, molecular mass standard; 2, ABC143A; 3, ABC143B; 4, ABC143C; 5, ABC143D; 6, ABC143E; 7, ABC143F; 8, ABC146; 9, ABC164; 10, MS6671.

a resistance island different from those of the other three plasmids, as it did not contain the *ermB*, *mph(A)*, *bla_{TEM-1B}*, *rmtB*, *bla_{NDM-5}*, or *sul1* genes, although it also harbored the *ltrA* gene, which is present in the other Emirati plasmids but notably absent from the Korean ones. On the other hand, the resistance islands of the three other plasmids exhibited high levels of similarity, especially in pCC1410-1 and pABC143C-NDM, which were, with the exception of the presence of *ltrA*, identical (Fig. 4).

DISCUSSION

Pan-drug-resistant *K. pneumoniae* infections have been reported since 2005 (14), and most recently, even clusters or outbreaks caused by the KPC-2 producer *K. pneumoniae* ST258 have been recorded (11, 15). In Greece, a single pan-drug-resistant *K. pneumoniae* ST147 isolate was also encountered among *K. pneumoniae* ST258 strains (11) representing the majority of local pan-drug-resistant isolates. Here, we report on a cluster of highly similar, pan-drug-resistant *K. pneumoniae* ST147 strains isolated from three cases in two emirates of the UAE. The short time between the isolation of strains from the first two patients in the same AD hospital and the association of the first patient with UAQ hospital A, where the third case was identified, make the connection likely. Nevertheless, the paucity of epidemiological data prevents us from substantiating this assumption. We assume that these isolates are also closely related to a pan-drug-resistant *K. pneumoniae* ST147 isolate from UAQ hospital B, MS6671, carrying three chromosomal copies of *bla_{OXA-181}* isolated a few months later (12). In addition to belonging to the same ST and having highly similar genotypes (Table 1) and similarities in their pulsed-field gel electrophoresis (PFGE) patterns (Fig. 1), our isolates also carried three copies of the OXA gene in fragments of their XbaI-restricted genomes of molecular masses identical to that of MS6671 (Fig. 2). In one strain, ABC143F, a fourth fragment of the PFGE pattern was also labeled, suggestive of the presence of another

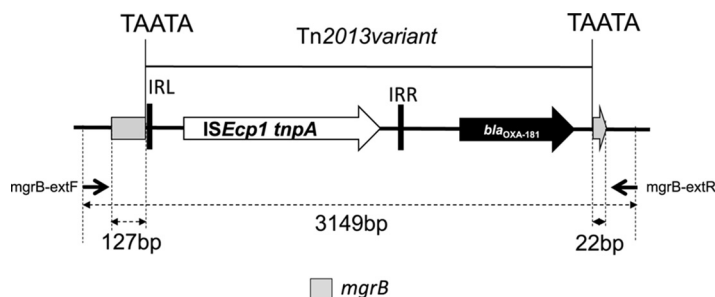


FIG 3 Structure of the *mgrB::bla_{OXA-181}* insert.

TABLE 3 Carbapenemase activity of the *mgrB::bla_{OXA-181}* structure cloned from ABC143C and MS6671

Strain	MIC (mg/liter) ^a					
	IMI	MEM	ERT	CAZ	CTX	AZT
DH5 α (pUC19)	<0.25	<0.25	<0.125	<0.25	<0.25	<0.25
ABC143C	>128	>128	>64	>128	>128	128
DH5 α (pCoxABC143C#13)	1	2	1	<0.25	<0.25	<0.25
MS6671	16	>128	>64	64	>128	>128
DH5 α (pCoxMS6671#6)	1	2	2	<0.25	<0.25	<0.25

^aIMI, imipenem; MEM, meropenem; ETP, ertapenem; CAZ, ceftazidime; CTX, cefotaxime; AZT, aztreonam.

copy of *bla_{OXA-181}*. As with isolate MS6671, colistin resistance of the current strains was also due to an *mgrB::bla_{OXA-181}* insertion. Furthermore, the backbone of the *bla_{NDM-5}*-containing plasmid pABC143C-NDM exhibited extensive similarities to that of pMS6671E. A possible explanation for the differences between these two plasmids might be that in MS6671, a recombination event between the chromosomally located class I integron and the integron located on the IncFII plasmid took place, resulting in the loss of the *ermB*, *mph(A)*, *bla_{TEM-1B}*, *rmtB*, *bla_{NDM-5}*, and *sul1* resistance genes. It was noteworthy, however, that this event, i.e., the loss of *bla_{NDM-5}*, did not change the susceptibility profile of *K. pneumoniae* MS6671, since its remarkable array of resistance genes (12) still made it pan-resistant. A similar scenario might have occurred in ABC143F, one of the isolates from the first patient, which lost the *rmtB* and *bla_{NDM-5}* genes. However, investigating this was beyond the scope of the present study.

Plasmid structures highly similar to pABC143C (pCC1410-1 and pCC1409-1 [GenBank accession no. [KT725788](#) and [KT725789](#)]) were previously revealed in two strains isolated in South Korea (5). Like members of the UAE cluster, they belonged to *K. pneumoniae* ST147 and both carried the *bla_{OXA-181}* and *bla_{NDM-5}* carbapenemases. Notably, the first strain (CC1409-1) was recovered from a patient who had been transferred in 2014 from an undisclosed hospital in Abu Dhabi to Seoul, South Korea. While CC1409-1 was still susceptible to colistin, the strain isolated 4 months later in the same hospital (CC1410-1) was resistant to polymyxins, although the basis of its colistin resistance has not been disclosed (13). Although these strains were not available to us for a more detailed comparison, on the basis of all the similarities between their genotypes and the nearly identical structures of their *bla_{NDM-5}*-carrying plasmids (Fig. 3), we surmise that these South Korean strains also closely related to this specific cluster of *K. pneumoniae* ST147 isolates.

Enterobacteriaceae possessing two carbapenemases have previously been observed worldwide (16) and in the region as well (6, 17, 18). It is of particular concern that representatives of the *K. pneumoniae* ST147 clone, well known for its epidemic potential (4, 6), not only are present in the UAE as dual-carbapenemase-producing isolates but also develop resistance to the last-resort antibiotic, colistin, by insertion of a functional carbapenemase gene into the *mgrB* gene. Apart from the earlier report on MS6671 (12), a similar *ISEcp1*-driven insertional mutation of *mgrB* in *K. pneumoniae* has been observed in a single isolate in France, but in that case, an extended-spectrum beta-lactamase gene (*bla_{CTX-M-15}*) was coded on the insertion, and the strain remained susceptible to an array of antibiotics (19).

It is not known to what extent the reduced susceptibility to chlorhexidine contrib-

TABLE 4 Complementation of the *mgrB::bla_{OXA-181}* mutation

Strain	MIC of colistin (mg/liter) ^a
ABC143C(pDG106)	16
ABC143C(pComgrB)	1
MS6671(pDG106)	16
MS6671(pComgrB)	1

^aColistin susceptibility tests were carried out in the presence of 100 μ M HgCl₂.

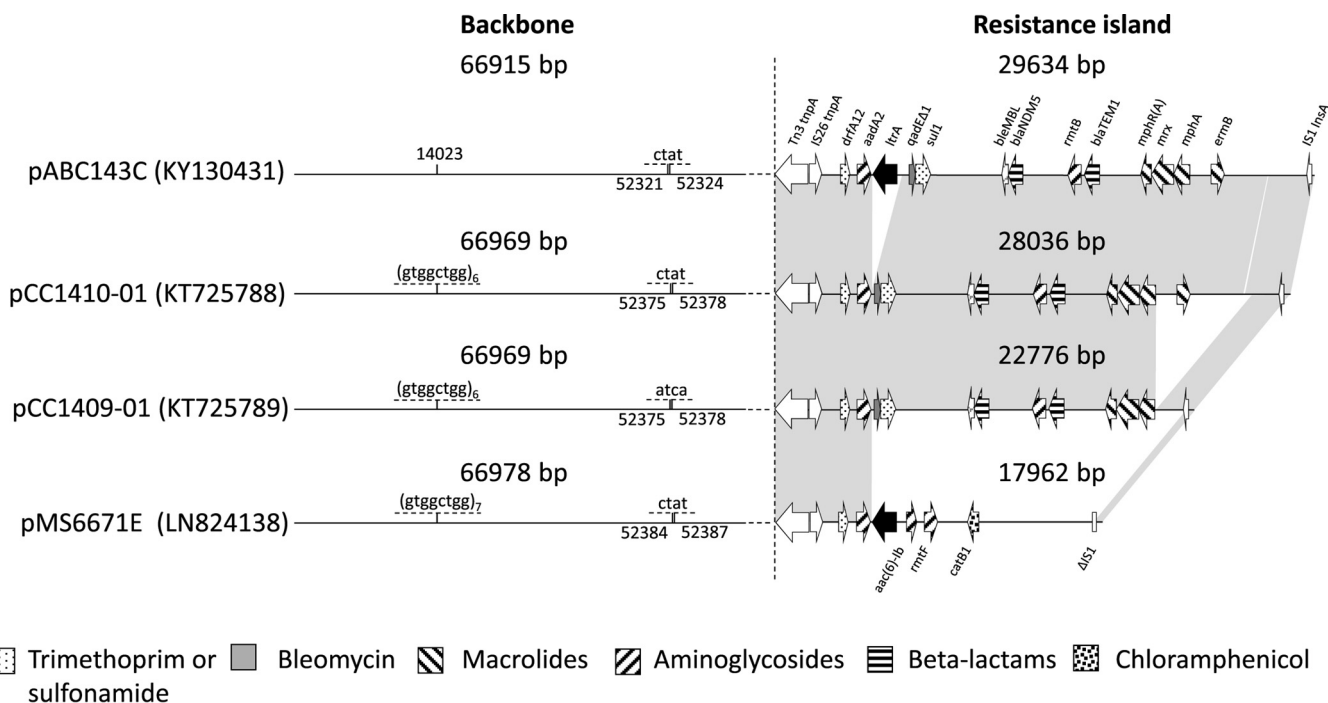


FIG 4 Structure of pABC143C. Note that the structures are represented disproportionately to their real size. The GenBank numbers of the plasmids are shown in parentheses.

uted to the spread or reoccurrence of this pan-drug-resistant *K. pneumoniae* ST147 clone in multiple hospitals. Carrying the *cepA* and *qacΔE* genes has been associated previously with such a phenotype (20). On the other hand, the insertional mutation leading to colistin resistance in our study was different from those recently described in colistin- and chlorhexidine-cross-resistant *K. pneumoniae* isolates, and the level of chlorhexidine nonsusceptibility did not reach the levels noted in that study (21).

Important limitations of the study are the lack of available more-detailed epidemiological data revealing the actual links and exploring the possible modes of transmission between the cases. Furthermore, as currently there are no nationwide antibiotic surveillance data and the laboratories are under no obligation to submit multidrug-resistant isolates for molecular typing, it is not known whether the cluster described represents its real size or additional such isolates are still present in the region.

Nevertheless, our data show that this pan-resistant *K. pneumoniae* ST147 clone, with a unique, *ISEcp1*-directed *bla*_{OXA-181} insertion in the *mgrB* gene, i.e., a single genetic event causing resistance to two agents used to treat severe Gram-negative infections, carries a considerable outbreak potential, as evidenced by its repeated occurrence in multiple hospitals, including possible international spread, over an extended period of time. Beyond enforced infection control measures, only extensive, national, and region-wide antimicrobial resistance surveillance with molecular typing investigations may carry the promise to contain the spread of such strains.

MATERIALS AND METHODS

Bacterial isolates. Strains were submitted to our laboratory for molecular typing according to the nonmandatory recommendation of the Health Authority Abu Dhabi regarding carbapenem-resistant *Enterobacteriaceae* (CRE) isolates. Strains were received with basic demographic and clinical information only, i.e., strictly without any patient identifiers. The isolates were preserved in duplicate at -80°C.

Antimicrobial susceptibility testing. Antibiotic susceptibility was routinely tested by microdilution according to the CLSI standards (22), while in the case of colistin, tigecycline, and fosfomicin, the EUCAST guidelines (23) were applied. Agar dilution was used to establish susceptibilities to chlorhexidine, benzalkonium, and irgasan.

Genotyping. The presence of virulence-related genes *magA*, *allS*, *mrkD*, *rmpA*, *kfuBC*, *fimH*, *ureA*, *uge*, *wabG*, *fyuA*, *iutA*, *ireA*, *iroN*, *cnf1*, *traT*, and *clpK* (24, 25), of those determining the capsular types (26), and of antibiotic and disinfectant resistance genes *bla*_{TEM}, *bla*_{CTX-M}, *bla*_{SHV}, *bla*_{PER}, *bla*_{AmpC}, *bla*_{NDM}, *bla*_{OXA-48-like}

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*bla*_{KPC}, *bla*_{VIM}, *bla*_{IMP}, *armA*, *rmtA*, *rmtB*, *rmtC*, *rmtD*, *rmtE*, *rmtF*, *qnrS*, *qepA*, *aac6'-1b-cr*, *emrE*, *qacDE*, *qacF*, *qacE*, *mdfA*, *ydgE*, *ydgF*, *sugE(c)*, *sugE(p)*, and *cepA* was tested by PCR as previously described (27). The allele of the OXA-48-like enzyme was determined by direct sequencing of the amplicon containing the *mgrB::bla*_{OXA} structure, while that of *bla*_{NDM} was revealed by PCR and direct sequencing with primers AS-ndm1 and AS-ndm2 (28).

Molecular typing. Isolates were compared by using rep-PCR (DiversiLab; bioMérieux) and applying a *Klebsiella* kit, according to the manufacturer's recommendation, and by XbaI-restricted genome digestion followed by pulsed-field gel electrophoresis (PFGE), as described previously (29). For hybridization of the PFGE gel, the amount of bacterial cells for plug preparation was doubled. PFGE patterns were analyzed with GelCompar II software (Applied Maths, Belgium) and by applying the unweighted pair group method with arithmetic mean (UPGMA) with the Dice similarity coefficient (SD) with a 1.5% position tolerance. The sequence type of the isolates was determined by multilocus sequence typing (MLST) (30) using the MLST webpage <http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html>.

Plasmid work. Extraction and electrophoresis of the plasmids were carried out by the method of Kado and Liu (31), using plasmids in *E. coli* 39R861 and *E. coli* V517 as molecular mass markers.

From *K. pneumoniae* ABC143C, the pABC143C-NDM-5 plasmid was transferred by conjugation into *E. coli* J53_{RAZ} as described previously (32). From the transconjugant, the plasmid was purified by using a plasmid maxiprep kit (Qiagen, Germany), and its complete sequence was determined commercially at the CCIB DNA Core Facility at Massachusetts General Hospital (Cambridge, MA). PCR mapping and direct sequencing were used to close the gaps between the contigs and to unambiguously determine the sequence of the plasmid. Antibiotic resistance genes on the assembled plasmid were identified by ResFinder 2.1 (33). The sequences were annotated using Sequin (<http://www.ncbi.nlm.nih.gov/Sequin>) and submitted to GenBank. The Inc type of the plasmid was deduced from its complete sequence (34).

Hybridization. Hybridization of the plasmid and PFGE gel transferred to Hybond N+ (Roche) membranes was carried out as described previously (32).

Cloning and complementing the *mgrB* gene with a *bla*_{OXA-181} insertion. To PCR clone the entire *mgrB* gene, with or without insertion, external primers (8) were used with additional BamHI plus CGCCGC overhangs. The *mgrB::bla*_{OXA-181} structure amplified was cloned into the BamHI-digested pUC19 and transformed into *E. coli* DH5 α (28), and transformants were selected on plates containing 2 mg/liter ertapenem.

To complement the *mgrB::bla*_{OXA-181} insertion, an intact *mgrB* gene was amplified from ABC193, a colistin-susceptible *K. pneumoniae* strain, and cloned into the BamHI site of pDG106, i.e., a vector containing the mercury resistance *merR* gene, obtained from the National Institute of Genetics, Japan (35). This construct was transformed (28) into the colistin-resistant wild-type strains ABC143C and MS6671, with selection of transformants on plates containing 100 μ M HgCl₂. The colistin susceptibilities of the transformants were compared to those of similarly prepared derivatives containing the pDG106 vector without the *mgrB* insert by using serial dilution of colistin sulfate with a constant concentration of 100 μ M HgCl₂ in order to maintain the plasmid in the derivatives.

Accession number(s). The sequences of the *mgrB* PCR product of ABC143C and pABC143C-NDM were deposited in GenBank under accession numbers KY426739 and KY130431, respectively.

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