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REVIEW

Plasmids carrying DHA-1 β-lactamases

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Abstract

The aim of this review is to provide an update on the plasmids mediating DHA-1 cephalosporinase in *Klebsiella pneumoniae*. These plasmids have been mainly found in this bacterium but not only. The first was isolated from *Salmonella* sp. in France in the early 1990s. They are currently reported worldwide. Bla_{DHA-1} beta-lactamase gene is usually co-expressed with many other antibiotic resistance genes such as extended-spectrum β-lactamases (bla_{CTX-M}-, bla_{SHV}-types), oxacillinases (bla_{OXA-1}, bla_{OXA-30}), penicillinases (bla_{TEM}-type), carbapenemases (bla_{OXA48}, bla_{KPC-2}), aminoglycosides (*aacA*, *aadA*, *armA*), fluoroquinolones (*qnrB4*, *aac6′-1b-cr*), and sulfonamide (*sul1*) resistance genes. Plasmids carrying DHA-1 cephalosporinase have different sizes (22 to 313 kb), belong to diverse groups of incompatibility (R, L/M, FII(k), FIB, A/C2, HI2, HIB), and are self-transferable or not. The multidrug resistance region consists of a mosaic structure composed of resistance genes, insertion sequences, composite transposon, and integrons.

Keywords Plasmids · AmpC · DHA-1 · Cephalosporinase · Antibiotic resistance · *Klebsiella pneumoniae*

Introduction

Production of β-lactamase is the main mechanism of resistance of *Enterobacteriaceae* to β-lactam antibiotics. Some *Enterobacteriaceae* naturally produce chromosome-encoded β-lactamase (penicillinase or cephalosporinase). Oxyimino-β-lactamins, such as cefotaxime or ceftazidime, have been developed to avoid these enzymes. Unfortunately, under antibiotic pressure, over-production of chromosomal AmpC beta-lactamases has occurred by mutation and conferred resistance to these molecules. Similar overproduction has been observed in *Enterobacter* sp., *Citrobacter freundii*, *Serratia marcescens*, *Morganella morganii*, and *Pseudomonas aeruginosa* [1]. During the early 1990s, plasmid-encoded AmpC cephalosporinases were reported in species that lack an inducible AmpC enzyme such as *Klebsiella pneumoniae*, *Escherichia coli*, and *Salmonella* sp. [2]. Subsequently, plasmid-mediated AmpC were observed worldwide. They are derivatives of *Enterobacter* sp. (ACT-1 and MIR-1), *C. freundii* (CMY-2), *M. morganii* (DHA type), *Hafnia alvei* (ACC-1), and *Aeromonas* sp. (CMY-1, FOX, MOX) [3]. The first DHA-1 cephalosporinase was described in a *Salmonella enterica* serovar Enteritidis strain isolated in Dhahran, Saudi Arabia, in 1992 [4]. It is the main inducible plasmid-mediated AmpC cephalosporinase (pAmpC).

Detection and susceptibilities

DHA-1 belongs to class C of the Ambler classification and to group 1 of the functional classification of Bush, Jacoby, and Medeiros [5, 6]. pAmpC confers resistance to all penicillins

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(alone or in combination with β-lactamase inhibitors) and to most cephalosporins (except cefepime), including cephamycin, but is inactive against carbapenems and cefepime. Unlike all other pAmpC, DHA-1 is always inducible because of the systematic presence upstream of bla_{DHA-1} of the regulatory gene *ampR*. This regulation explains the antagonism between cefoxitin, clavulanate, and carbapenem, and oxyiminocephalosporins. Such antagonism can be a strong indicator of DHA-1 production in species that do not possess chromosomal-inducible AmpC, such as *E. coli* or *K. pneumoniae*. Some phenotypical tests have been developed to detect AmpC production in non-AmpC-producing species, using cephalosporins alone or in combination with cloxacilline or boronic acid, which are inhibitors of AmpC [7–10]. However, these tests are unable to precisely identify DHA-1 producers and they are unusable on AmpC-producing species such as *E. coli*, *Enterobacter* sp., and *C. freundii*. Molecular methods are the reference strategy for DHA-specific detection, of which Pérez-Pérez multiplex PCR is the most widely used [11].

History (Table 1)

The first plasmid-mediated DHA β-lactamase was identified in a strain of *S. enterica* serovar Enteritidis from stool samples of a 62-year-old patient with lung carcinoma in Dhahran (Saudi Arabia) in 1992 [4]. A strain of *K. pneumoniae*-producing DHA-1 was isolated from urine specimens in Los Angeles (USA) in 1994 [12] and another one in Miami (USA) between 1996 and 2000 [13]. Two strains of *S. enterica* serovar Senftenberg-producing DHA-1 were isolated in London (UK), in 1996 and 1999. They carried plasmids of ca. 98 and 99 kDa that were self-transferable [14]. In France, the first *K. pneumoniae*-producing DHA-1 was isolated in Paris in 1998 from blood cultures. The plasmid was self-transferable. Nine other *K. pneumoniae* and one *Klebsiella oxytoca*-producing DHA-1 strains were later isolated between 1999 and 2003 (five in 2003). Only two were not self-transferable [15]. Fifty-four isolates of *K. pneumoniae* and 2 isolates of *E. coli*-producing DHA-1 β-lactamase were identified from blood cultures between 1993 and 2005 in Seoul (Korea). All isolates coproduced *qnrB4* (*qnr* genes are plasmid-mediated quinolone resistance) and some also produced other enzymes (CTX-M types, TEM-52, SHV-12) [16, 17]. Ten isolates of *K. pneumoniae*-producing DHA-1 β-lactamase were identified between January 1999 and September 2001 in Taiwan. The bla_{DHA-1} gene was located on 70-kb plasmids that were not self-transferable [18]. A 3-year-old girl with diarrhea and fever was admitted to hospital in October 2002 in Korea. A *Salmonella enterica* serotype Montevideo DHA-1 positive was isolated from blood and stool specimens. The plasmid was self-transferable [19]. Thirty-six DHA-1-producing *K. pneumoniae* that co-expressed ESBL enzymes (CTX-M and/or SHV) were collected between January 1999 and June 2002 in Taiwan [20]. From May to July 2004, 11 DHA-1- and SHV-12 ESBL-producing *K. pneumoniae* were collected in a Korean hospital [21]. Ninety-nine DHA-1-producing *K. pneumoniae*, 13 DHA-1-producing *E. coli*, and 1 DHA-1-producing *Enterobacter cloacae* were collected during 2004 to 2006 in Kyungpook (Korea) [22]. As elsewhere, some of the isolates co-expressed ESBL enzymes (CTX-M and/or SHV). Further, 98/99 isolates of DHA-1-producing *K. pneumoniae* and 100% isolates of DHA-1-producing *E. coli* were positive for *qnrB4*. Then, 89/99 of *K. pneumoniae* and 7/13 of *E. coli* isolates were positive for *armA*, a plasmid-mediated 16S rRNA methylase gene. Conjugation was successful for 38 DHA-1-producing *K. pneumoniae*, 3 DHA-1-producing *E. coli*, and 1 DHA-1-producing *E. cloacae*. Thirty-five transconjugants carried *qnrB4*, bla_{DHA}, bla_{SHV-12}, and *armA*; two transconjugants carried *qnrB6*, bla_{DHA-1}, and *armA*. Nine plasmids were chosen for further studies, which revealed a large plasmid of about 180 kb belonging to the FIIA incompatibility (Inc) type [22]. In another study, genes *qnrB4* and *qnrS* were found in the same strain of *K. pneumoniae* but on different plasmids. The strain was isolated in 2005 in China [23]. In southern Taiwan, 20 isolates of flomoxef-resistant ESBL-*K. pneumoniae*, all carrying the bla_{DHA-1} gene, were identified between March 2004 and November 2005. Bla_{CTX-M} genes were found in 18 of the strains: *qnr*, *aac(6′)-Ib-cr*, and *armA* genes were respectively detected in 100, 61, and 78% of the isolates. Conjugation was successful for only four strains. Genes *qnrB4*, *aac(6′)-Ib-cr*, *armA*, and bla_{DHA-1} were on the same self-transferable CTX-M-carrying plasmids. They belonged to the FIIA Inc group [24]. Plasmid pKP048, belonging to IncF, was detected in carbapenem-resistant *K. pneumoniae* in China in 2006. It carried bla_{KPC-2}, bla_{DHA-1}, *qnrB4*, and *armA*, and was self-transferable [25]. Plasmid p1220-CTXM, a pKP048-related IncFII(k) plasmid, was recently sequenced in China; it carried $bla_{CTX-M-14}$ and *qnrB4* [26]. A hospital in the northwest of Belgium recorded 22 clinical isolates of *K. pneumoniae* expressing plasmid-mediated DHA-1 AmpC. and chromosomal SHV-11 beta-lactamases between August and December 2006 [27]. Thirty *Enterobacteriaceae* (15 *E. coli*, 10 *K. pneumoniae*, 4 *K. oxytoca*, and 1 *Proteus mirabilis* strains) encoding bla_{DHA-1} or bla_{DHA-1} plus $bla_{CTX-M-14, -15}$ were collected between 2005 and 2007 in Barcelona, Spain. All isolates co-harbored bla_{DHA-1} and *qnrB* genes on the same plasmid. Conjugation was possible for 26/30 isolates, of which all but one belonged to the L/M group [28]. Twenty-six DHA-1-producing *K. pneumoniae* were isolated between June 2007 and January 2008 in Barcelona, Spain. Three of them also had $bla_{CTX-M-15}$ and *aac(6′)-Ib-cr* and 25 contained *qnrB*. Transconjugants were obtained with one isolate of each pulsed-field gel

Table 1 Summary of history of DHA-1 diffusion

Years/reference	City/country	Species	Plasmid size	Inc group	ST	β-Lactam resistances	Other antibiotic resistances
1992 [4]	Dharan/Saudi Arabia	*S. enteritidis*					
1994 [12]	Los Angeles/USA	*K. pneumoniae*					
1996–2000 [13]	Miami/USA	*K. pneumoniae*					
1996–1999 [14]	London/UK	*S. senftenberg*	98–99 kDa		Yes		
1998 [15]	Paris/France	*K. pneumoniae*			Yes		
1999–2003 [15]	Paris/France	*K. pneumoniae* *K. oxytoca*			Yes (9/11)		
1993–2005 [16, 17]	Seoul/Korea	*K. pneumoniae* *E. coli*				CTX-M-14 TEM-52, SHV-12	*qnrB4*
1999–2001 [18]	Taiwan	*K. pneumoniae*	70 kb		No		
2002 [19]	Korea	*S. montevideo*			Yes		
1999–2002 [20]	Taiwan	*K. pneumoniae*				SHV-type, TEM-1, CTX-M-3, CTX-M-14	
2004 [21]	Korea	*K. pneumoniae*				SHV-12	
2004–2006 [22]	Kyungpook/Korea	*K. pneumoniae* *E. coli* *E. cloacae*	180 kb	FIIA, FIA (ST)	V	CTX-M-3, CTX-M-15 SHV-12	*qnrB4*, *armA* or *qnrB6*, *armA*
2005 [23]	China	*K. pneumoniae*					*qnrB4* and *qnrB6* on different plasmids
2004–2005 [24]	Taiwan	*K. pneumoniae*		FIIA (ST)	V	CTX-M-3, CTX-M-14 SHV-5, SHV-12	*qnrB4*, *qnrA*, *aac(6')-Ib-cr*, *armA*
2006 [25]	China	*K. pneumoniae*	151 kb (pKP048)	F	Yes	KPC-2	*qnrB4*, *armA*
2006 [27]	Belgium	*K. pneumoniae*				SHV-11	
2005–2007 [28]	Barcelona/Spain	*E. coli* *K. pneumoniae* *K. oxytoca* *Proteus mirabilis*		L/M	Yes (26/30)	CTX-M-14	*qnrB*
2007–2008 [29]	Barcelona/Spain	*K. pneumoniae* ST17, ST13, ST427, ST416, ST37, ST326, ST428	180 kb 120 kb	L/M FII	Yes	CTX-M-15	*qnrB*, *aac(6')-Ib-cr*
2007 [30]	China	*S. thompson*				TEM-1b	
2007 [31]	Vancouver/British Columbia (Canada)	*C. freundii*	22–24 kb			OXA-1, OXA-10	*qnrB4*, *catB3*, *sul1*, *dfrA14*, *cmlA5*, *aadA1*, *aac(6')-Ib-cr*
2008 [32]	Tortosa/Spain	*K. pneumoniae* ST483				OXA-1	*qnrB4*, *aac-(6')-Ib-cr*, *catB3*, *arr3*, *sul1*
2008 [33]	Shanghai/China	*K. pneumoniae* ST11		FII	Yes	SFO and TEM-1 on different plasmids	*qnrB4*, *armA*
2008 [34]	Paris/France	*K. pneumoniae* ST11, SHV11	61 kb (pKPS30)	R	No	OXA-30	*qnrB4*, *aac(6')-Ib-cr*, *catB3*, *tetA*, *arr-3*, *sul1*, *mphA*
2006–2010 [36]	Paris/France	*K. pneumoniae* ST48, ST1263 *K. oxytoca* *E. coli*	85 kb	L/M HI2	Yes (8/14)	SHV-12, TEM-1, OXA-30	

Table 1 (continued)

Years/reference	City/country	Species	Plasmid size	Inc group	ST	β-Lactam resistances	Other antibiotic resistances
2008–2010 [37]	Madrid/Spain	*K. pneumoniae* ST11, SHV11	50 kb	R	No		*qnrB4*, *armA*
2009 [38]	Hong Kong	*E. coli*	89 kb (pNDM-HK)	L/M	Yes	TEM-1	*aac2*, *armA*, *sul1*, *mel*, *mph2*
2009 [39]	Serres/Greece	*K. pneumoniae* ST11	140 kb	FII	No	OXA-1 OXA-162 on a different plasmid (62 kb, IncL/M)	
2010 [40]	China	*E. aerogenes*	56 kb		Yes	KPC-2	
2011–2012 [41]	Paris/France	*K. pneumoniae* ST274	254 kb (pENVA)	FIB, HIB	Yes	CTX-M-15, TEM-1	*aacA2*, *aadA1*, *qnrB4*, *dfrA15*, *sul1*
2009–2013 [42]	Hungary	*K. pneumoniae*				CTX-M-15	Colistin (*mgrB*)
2016 [43]	Japan	*K. pneumoniae* ST37				SHV12	Colistin (*mgrB*) Tigecycline (*ramR*)
2017 [26]	China	*K. pneumoniae*	137 kb (p1220-CTXM)	FIIk		CTX-M-14	*qnrB4*, *sul1*

ST self-transferable, *V* variable

electrophoresis (PFGE) profile. The bla_{DHA-1} gene was present in a plasmid of 180 kb belonging to the L/M Inc group. Exceptionally, one gene was located in a plasmid of 120 kb. The FII replicon was also detected. The plasmids were transferable and the strains belonged to sequence type (ST) 17, ST13, ST427, ST416, ST37, ST326, and ST428 [29]. In September 2007, a strain of *S. enterica* Thompson co-harboring bla_{DHA-1} and bla_{TEM-1b} was isolated from the stool sample of a pediatric inpatient (aged 3 years) with diarrhea in China [30]. Three strains of *C. freundii* were isolated from wastewater treatment plants in Vancouver, British Columbia (Canada), in June 2007. They carried large plasmids (> 60 kb) with resistance genes such as *qnrB4*, *aac*(*6′*)*-Ib-cr*, bla_{DHA-1}, and bla_{OXA-1} or bla_{OXA-10} [31]. Two *K. pneumoniae* isolates carrying bla_{DHA-1} and *qnrB4* genes were identified in 2008 in Tortosa, Catalonia (Spain). They belonged to the same MLST, ST483, and harbored the *intI1*, bla_{DHA-1}, bla_{OXA-1}, bla_{SHV-1}, *aac*(*6′*)*-Ib-cr*, *qnrB4*, and *qnrS2* genes [32]. A SFO-1-producing strain of *K. pneumoniae* isolated in 2008 in Shanghai (China) harbored a large DHA-1-bearing IncFII plasmid in a ST11 clone (SFO-1 is an ESBL enzyme). The plasmid also carried a *qnrB4* gene [33]. *K. pneumoniae* KPS30 strain (ST11) was isolated from urine in 2008 in Paris (France). KPS30 carries the ß-lactamase genes bla_{SHV-11}, bla_{OXA-30}, and bla_{DHA-1}. pKPS30 is a 61,228-bp plasmid of the IncR group. It carries bla_{OXA-30} and bla_{DHA-1} genes and *tetA*, *aac6′-Ib-cr*, *catB3*, *arr-3*, *sul1* (two copies), and *mph*(*A*) genes. It was not transferable [34]. At the same time, a similar plasmid of 57,382, KpQ3 was isolated in Madrid (Spain) in a ST37 *K. pneumoniae* strain [35]. Seventeen *K. pneumoniae* strains producing DHA-1 were collected between 2006 and 2010 in Paris (France). ST48 and ST1263 were predominant and the plasmids belonged to IncL/M or IncHI2 groups; 8/14 were transferable [36]. Seven *K. pneumoniae* strains were isolated from dogs and cats in Spain between 2008 and 2010. All isolates were typed as ST11 and had SHV-11 ß-lactamase. The IncR plasmids of 50 kb carried bla_{DHA-1}, *armA*, and *qnrB4* genes and were not transferable [37]. A carbapenemase NDM-1 (for New Delhi metallo-carbapenemase) *E. coli* strain was isolated in October 2009 in Hong Kong. The plasmid named pNDM-HK, 88,803 bp in size, was self-transferable and belonged to the IncL/M group. It also harbored genes associated with resistance to ß-lactams (bla_{TEM-1}, bla_{DHA-1}), aminoglycosides (*aac2*, *armA*), sulfonamides (*sul1*), and macrolides (*mel*, *mph2*) [38]. A ST11 *K. pneumoniae* strain co-expressing the OXA-162 carbapenemase and DHA-1 was isolated in Serres (Greece) in 2010 [39]. The $bla_{OXA-162}$ gene was harbored on a plasmid of 62 kb belonging to IncL/M while the bla_{DHA-1} gene was carried on an IncFII plasmid of 140 kb. The plasmids were not self-transferable [39]. A carbapenem-resistant *Enterobacter aerogenes* was isolated from the sputum of a 69-year-old patient in China in July 2010. The strain harbored a plasmid of

56 kb carrying carbapenemase KPC-2, DHA-1, and TEM-1; the plasmid was self-transferable by conjugation [40]. Fourteen ST274 *K. pneumoniae* were recovered from dogs, cats, sheep, and a hedgehog between July 2011 and June 2012 in Paris (France). They harbored a plasmid of 253,984 bp named pENVA carrying $bla_{CTX\text{-}M\text{-}15}$, $bla_{TEM\text{-}1}$, $bla_{DHA\text{-}1}$, *aacA2*, *aadA1*, *qnrB4*, *dfrA15*, and *sul1* (two copies) genes. Two replicons, those of IncFIB and IncHIB-like plasmids, were found [41]. Between December 2009 and December 2013, 312 DHA-1-producing *K. pneumoniae* strains of ST11 were isolated in Hungary. The $bla_{CTX\text{-}M\text{-}15}$ was present in 90% of isolates and three isolates were also resistant to colistin by inactivation of *mgrB* [42]. In July 2016, a colistin- and tigecycline-resistant *K. pneumoniae* strain was isolated from a dog in Japan. Insertional inactivation of *mrgB* and *ramR* by IS10R could have been involved in these resistances. The strain belonged to ST37, carried $bla_{SHV\text{-}12}$ and $bla_{DHA\text{-}1}$, and was positive for *qnrB* and *armA* [43].

Complete plasmids (Table 2)

Twenty-one complete DHA-1 plasmids are available on GenBank (www.ncbi.nlm.nih.gov/genbank/). They mainly belong to the *Enterobacteriaceae* family: 13 were found in *K. pneumoniae*, 2 in *E. coli*, 3 in *C. freundii*, 1 in *Raoultella ornithinolytica*, and 1 in *Cronobacter sakazakii* strains. One plasmid was found in a strain of *Vibrio cholerae*. We looked at their Inc groups with PlasmidFinder [44] and at their profiles of resistance with Card [45]. They belong to different Inc groups: R (2), A/C2 (4), R and A/C2 (1), L/M (1), FII(k) (2) , FII(k) and R (2), R and FII(k) (2), HIB and FIB(Mar) (1), HI2 (1), H12A and H112 (1). Their sizes varied from ca. 22 to 312 kb. They carried many antibiotic resistance genes: to beta-lactams with bla_{OXA}-type, bla_{SHV}-type, $bla_{CTX\text{-}M}$-type, bla_{TEM}-type, bla_{IMP}-type, bla_{NDM}-type, bla_{KPC}-type, $bla_{DHA\text{-}1}$, to aminoglycosides with *aph*(3′)-type, *aac*(6′)-*Ib-cr*, *aadA1*, *aacA4*, *strA*, *strB*, *armA*, to fluoroquinolones with *aac*(6′)-*Ib-cr* and *qnrB4*, to phenicols with *catB3*, *floR*, *cmlA*, to rifampicine with *arr*, to sulfonamides with *sul1* and *sul2*, to trimethoprim with *dfrA*, to tetracyclines with *tetA*, and to macrolides with *mph*. In some of them, $bla_{DHA\text{-}1}$ and *ampR* genes were part of a ISCR1-linked resistance gene region associated with a class 1 integron. Fifteen of the 21 plasmids had an ISCR1 element. The genetic organization of the multidrug resistance regions is described in Table 2.

Patric database (Table 3)

We looked in the Patric database [46] for genomes of *Klebsiella* strains that were resistant to cefoxitin by synthesis of a class C beta-lactamase from the 'DHA/MOR' family. We found 26 genome assemblies and 1 complete plasmid (data supplement). The strains were collected from urine, blood, sputum, rectum, canal, pus, and river water between 2008 and 2017 in the USA, Europe, Asia, and Africa. The genomes were mainly sequenced by Illumina technology. We looked for the presence of plasmids with PlasmidFinder [44], for antibiotic resistance genes with Card [45], and for ST, *wzi*, and virulence genes on the Pasteur MLST site (http://bigsdb.pasteur.fr/). Contigs were compared with known DHA-1 plasmids by Mauve [47].

The strains harbored several plasmids with two to six different Inc groups. Plasmids related to pKPS30 were the most frequently recovered (ten times). pKPS30 plasmid of the Inc-R group was not self-transferable by conjugation [36]. It was associated with *K. pneumoniae* strains of ST11 [36] and ST37 [35]. It was also found associated with other STs such as ST895, ST29, and ST1307. Sequence types ST11, ST37, ST29, and ST895 were the most frequently found. No homology was found for eight plasmids. Five strains produced carbapenemase (NDM-type or OXA-48).

Most of the strains were of classical pathogenesis with type 3 fimbriae (*mrk* operon). Usually, hypervirulent isolates belong to serotypes K1 or K2. In this study, just one strain was of serotype K2 but possessed only the iron transport *kfu* operon as extra virulent genes and therefore could not be considered as hypervirulent. Four strains had a yersiniabactin, which is a siderophore that increases the virulence of the strain [48]. One strain of serotype K54 could be considered as hypervirulent with several siderophores (yersiniabactin and aerobactin [49]) and the presence of RmpA, a regulator of the mucoid phenotype [50]. The *K. pneumoniae* AR_048 strain (Accession: CP021950.1) of ST11 was sequenced by PacBio technology with a genome coverage of 120. The isolate belongs to a collection of carbapenemase-producing *Enterobacteriaceae*. It has four plasmids, one of which is a DHA-1 plasmid of 176,349 bp: tig00000169_pilon (Accession: CP021952.1). PacBio technology allows the complete sequencing of plasmids. DNAplotter (Sanger Institute) [51] was used to construct a schematic plasmid map (Fig. 1). Plasmid tig00000169_pilon belongs to the IncA/C2 group. The backbone part included genes for replication, transfer, stabilization, and transfer: the plasmid, therefore, is self-transferable by conjugation. Several antibiotic genes were found in the variable region: resistant to ß-lactams with $bla_{SHV\text{-}11}$, $bla_{DHA\text{-}1}$, $bla_{CMY\text{-}6}$, $bla_{NDM\text{-}1}$; resistant to aminoglycosides with *aac*(6′)-*Ib-cr*, *aac*(3)-*IId*, *rmtC*; resistant to fluoroquinolones with *aac*(6′)-*Ib-cr* and *qnrB9*; and to sulfonamides with *sul1*. The co-occurrence of two genes of class C ß-lactamase has already been reported [52]. However, the presence of $bla_{SHV\text{-}11}$, $bla_{DHA\text{-}1}$, $bla_{CMY\text{-}6}$, and $bla_{NDM\text{-}1}$ on the same plasmid is very surprising. Genes $bla_{DHA\text{-}1}$ and $bla_{NDM\text{-}1}$ belong to a class 1 integron of sul1-type with ISCR1 (see below) (Fig. 2).

Table 2 Complete DHA-1 plasmids from GenBank (https://www.ncbi.nlm.nih.gov/genbank/)

Incompatibility group (PlasmidFinder)	Plasmid name	Size (bp)	Antibiotic resistances (Card)	Genetic organization
K. pneumoniae				
R	pKPS30 [36]	61,228	bla_{OXA-1}, bla_{DHA-1} *aac(6')Ib-cr*, *qnrB4* *aph(3')-Ia*, *mph(A)* *catB3*, *arr-3*, *sul-1* (× 2), *tet(A)*	Typical class 1 integron with ISCR1 (Fig. 3a)
R ST37	PYDC676 [33]	50,182	bla_{DHA-1}, *strA*, *strB* *armA*, *qnrB4* *mph(E)*, *msr(E)*, *sul1*	pKP048 [25] (ISCR1 element) with operon *psp* between bla_{DHA-1} and *qnrB4*
A/C2	pKp55	215,528	bla_{OXA-31}, bla_{DHA-1} bla_{IMP-26}, *aac(3)-IId* *aacA4*, *aac(6')Ib-cr* *mph(A*, *catB3* (× 2) *sul1* (× 2), *sul2*, *arr-3* *floR*	ISCR1 with IS26 (5′-CS) and *tniB* (3′-CS)
A/C2	pHM881QN	160.687	bla_{OXA-10}, bla_{DHA-1} $bla_{SHV-126}$, *aadA1* *aacA4*, *aac(6')Ib-cr* *qnrB4*, *sul1*(×2), *sul2* *cmlA1*, *dfrA14* *arr-2*, *floR*	Typical class 1 integron (VR1: *dfrA14*, *arr-2*, *cmlA7*, *oxa-10*, *aadA1*), ISCR1 (VR2: *sap* operon, *qnrB4*, *psp* operon, bla_{DHA-1}, *ampR*) with truncated 3′-CS: only *sul1* followed by *tniB*
A/C2	pIMP-PH114	151,885	bla_{DHA-1}, bla_{IMP-4} *aacA4*, *aac(6')Ib-cr* *catB3*, *sul1*, *sul2*	Typical class 1 integron (VR1: bla_{IMP-4}, *qacG2*, *aacA4*, *catB3*), ISCR1 (VR2: bla_{DHA-1}, *ampR*), no 3′-CS, *tnii*
R-A/C2	pTR2	133,650	bla_{OXA-9}, bla_{DHA-1} bla_{NDM-1}, bla_{SHV-11} *aadA1*, *aac(3)-IId* *strA*, *strB*, *aac(6')Ib-cr*, *mph(E)*, *msr(E)*, *cmlA1*, *arr-2* *sul1*(× 2), *sul2*, *armA*	IS 26-composite transposon: bla_{NDM-1}, bla_{DHA-1}, *ampR*, *armA*, *sul1*
L/M	pNDM-OM	87,185	bla_{DHA-1}, bla_{TEM-1B} bla_{NDM-1}, *aac(3)-IId* *armA*, *mph(E)*, *msr(E)*, *sul1*	IS26, bla_{NDM-1}, ble_{MBL}, bla_{DHA-1}, *ampR*, *sul1*, ISCR1, ISEc28, *armA*, ISEC29, *msrE*, *mphE*
FII	pM16-13	111,951	bla_{OXA-1}, bla_{DHA-1} bla_{TEM-1B}, $bla_{CTX-M-15}$ *aph(3')-Ia* *aac(6')-Ib-cr* *qnrB4*, *mph(A)* *catB3*, *sul1* (×2), *arr-3* *tet(A)*	pRBDHA [15] (Fig. 3a)
FII(K)	pNDM-1saitama01	49,441	bla_{DHA-1}, bla_{NDM-1} *aph(3')*-VIa *mph(E)*, *msr(E)*, *arr-2* *sul-1*, *dfrA14*	PKP048 [25] with ble_{MBL}, bla_{NDM-1}, ISAba125 in 3′ after bla_{DHA-1}
FII(K)-R	p1220-CTXM [26]	137,060	bla_{DHA-1}, $bla_{CTX-M-14}$ *qnrB4*, *sul1*	TnpA, $bla_{CTX-M-14}$, ISEcp1, ISCR1, IS6100, *sul1*, qacEΔ1, *ampR*, bla_{DHA-1}, *psp* operon, *qnrB4*, *sap* operon, IS26
	pKP048 [25]	151,188	bla_{DHA-1}, bla_{KPC-2} *qnrB4*, *mph(E)*, *msr(E)*, *sul1*, *armA*	Fig. 3b
HIB, FIB(Mar)	pENVA [41]	253,984	bla_{DHA-1}, bla_{TEM-1B} $bla_{CTX-M-15}$, *aadA1* *aac(3)-IIa*, *qnrB4* *sul1*(× 2), *tet(A)*, *dfrA15*	VR1: *dfrA15*, *aadA*, IS26 instead of ISCR1, VR2: *qnrB4*, psp operon, bla_{DHA-1}, *ampR*
UN	pRJA166a	230,606	bla_{DHA-1}, $bla_{SHV-126}$ *qnrB4*, *sul1*	PhS7 [23] (Fig. 3a)

Table 2 (continued)

Incompatibility group (PlasmidFinder)	Plasmid name	Size (bp)	Antibiotic resistances (Card)	Genetic organization
E. coli				
FIB, F, FII	Plasmid A	184,614	bla_{DHA-1}, *qnrB4* *mph(A)*, *sul1*	*ampR*, bla_{DHA-1}, *psp* operon, *qnrB4*
L/M	pNDM-HK [38]		= PNDM-OM	IS26, bla_{NDM-1}, ble_{MBL}, bla_{DHA-1}, *ampR*, *qacEΔ1*, *sul1*, ISCR1, tpnU, *armA*, tpnD, *mel*, *mph2*, IS26
C. freundii				
UN	pCFI-1 [31]	23,574	bla_{OXA-1}, bla_{DHA-1} *aac(6')Ib-cr* *qnrB4*, *catB3*, *arr-3* *sul1*(× 2)	pRBDHA [15]
UN	pCFI-2 [31]	24,555	bla_{OXA-10}, bla_{DHA-1} *aadA1*, *qnrB4* *cmlA1*, *arr-2* *sul1*(× 2), *dfrA14*	pRBDHA VR-1: *dfrA14*, *arr-2*, *amlA5*, bla_{OXA-10}, *aadA1*
UN	pCFI-3 [31]	22,634	bla_{OXA-1}, bla_{DHA-1} *aac(6')Ib-cr*, *qnrB4* *catB3*, *arr-3*, *sul1*(× 2)	pRBDHA
R. ornithinolytica				
HI2	pYNKP001-drfA	234,154	bla_{DHA-1}, *aadA16* *qnrB4 arr-3*, *sul1*(× 2) *dfrA21*	Typical class 1 integron (VR1: *arr-3*, *dfrA27*, *aadA16*), ISCR1 (VR2: *sap* operon, *qnrB4*, *psp* operon, bla_{DHA-1}, *ampR*)
V. cholerae				
A/C2	pNDM-116-17	167,832	bla_{OXA-10}, bla_{DHA-1} bla_{NDM-1}, *aadA1* *mph(E)*, *msr(E)*, *cmlA1*, *arr-3*, *sul1*	IS26, bla_{NDM-1}, ble_{MBL}, tnpF, bla_{DHA-1}, *ampR*, *qacEΔ1*, *sul1*, ISCR1, tpnD, *msr(E)*, *mph(E)*, IS26
C. sakazakii				
HI2A, HI2	P505108-MDR	312,880	bla_{DHA-1}, bla_{TEM-1B} bla_{DHA-1}, bla_{SHV-12} *aacA4*, *aph(3')-Ia* *strA*, *strB* *aac(6')Ib-cr*, *qnrB4* *catA2*, *sul1*(× 2), *tet(D)* *dfrA18*	pMPDHA with *qnrB4* between *psp* operon and *sap* operon

UN unknown

Genetic organization (Fig. 3)

Isolates carrying ISCR1

The bla_{DHA-1} gene has been observed in a particular class 1 integron of *sul1*-type which contains two partial copies of the 3′-conserved segment *qacEΔ1sul1* surrounding a common region ISCR1 and antibiotic resistance genes [53, 54]. Different antibiotic resistance genes (VR1) have been detected between the *int* gene, encoding the integrase, (the 5′-conserved segment) and the first *qacEΔ1sul1* segment (3′-conserved segment). For example, *aadB* and *aadA2* were detected in the integron from pKp760 plasmid [15]; *dhfr*, *arr2*, *cmlA7*, and a fused gene cassette bla_{OXA10}-*aadA1* were detected in the integron from pRDDHA plasmid (the *clmA7* and bla_{OXA10} genes were inactivated by insertion sequences IS); *aac(6′)-Ib-cr*, bla_{OXA-30}, *catB3*, and *arr-3* were detected in the integron from pRBDHA and pKPS30 plasmids [36]; and *aac(6′)-II*, *ereA2*, *aac*, and *arr* were detected in the integron from pMPDHA plasmid (*ereA2* was interrupted by IS1247) [15]. The bla_{DHA-1} and *ampR* region was mobilized from the *Morganella morganii* chromosome and inserted downstream of ISCR1 [15]. This region was followed by the second *qacEΔ1sul1* segment (3′-conserved segment). In pMPDHA, IS26 followed the second *qacEΔ1sul1* segment, whereas a partial *tniB* was found in pRDDHA and pKp760. In pRBDHA, the end of the second *qacEΔ1sul1* segment was deleted by IS4321. In pMPDHA, pRBDHA, pHS7, and pKPS30, genes of the *sap* operon (ABC transporter family) and genes of the *psp* operon (phage shock proteins) were found downstream of ISCR1 and upstream of *ampC* [15, 23, 36]. In pRBDHA and pHS7, a *qnrB4* gene was inserted

Table 3 Analysis of genomes of *K. pneumoniae*-producing DHA-1 from Patric database

Plasmids (PATRIC)	Contigs	Inc (PlasmidFinder)	Card (penicillinase, main traits)	ST (Pasteur)	Plasmid homology	Virulence genes (Pasteur)	wzi gene (Pasteur)	K serotypes (Pasteur)
tig00000169_pilon	1	A/C2	SHV-11, CMY-6, NDM-1	ST11	Complete plasmid	iutA	/	/
FLXW01.1.fsa_nt	43	R, FII(K), FIB(K)	SHV-11	ST895	pKPS30	mrkABCDFHIJ	173	/
FLXX01.1.fsa_nt	46	R, FIB(K), FII(K)	SHV-11	ST895	pKPS30	mrkABCDFHIJ	173	/
FLXO01.1.fsa_nt	41	R, FII(K), FIB(K)	SHV-11	ST895	pKPS30	mrkABCDFHIJ	173	/
FLXP01.1.fsa_nt	43	R, FII(K), FIB	SHV-11	ST895	pKPS30	mrkABCDFHIJ	173	/
FLXV01.1.fsa_nt	85	R, FII(K), FIB	SHV-11	ST895	pKPS30	mrkABCDFHIJ	173	/
FLXS01.1.fsa_nt	43	R, FII(K), FIB	SHV-11	ST29	pKPS30	mrkABCDFHIJ	173	/
AWOM01.1.fsa_nt	84	R, FIB(K)	SHV-11	ST37	pKPS30	mrkABCDFHIJ	110	/
AWON01.1.fsa_nt	148	R, FIB(K)	SHV-11	ST37	pKPS30	mrkABCDFHIJ	110	/
NQEO01.1.fsa_nt	100	R, FIB(pKPHS1), FII(K)	SHV-11	ST1307	pKPS30	mrkABCDFIJ, kfuABC	202	/
NNCC01.1.fsa_nt	154	R, FIB(K), FII(K), L/M (pOXA-48)	SHV-11, OXA-48	ST11	pKPS30	mrkABCDHIJ, ybtA, irp1, 2, fyuA	50	K15, 17, 50, 51, 52
LWLM01.1.fsa_nt	81	R, FII(K), X9,	SHV-126	ST29	p1220-CTXM	mrkABCDFHIJ, rmpA, rmpA2, ybtAEPQSTUX, iroBCN, irp2, iucABCD, iutA	115	K54
FLVS01.1.fsa_nt	34	R, FII(K), FII(K), FIB(K)	SHV-83	ST29	p1220-CTXM	mrkABCDFHIJ, fyuA, irp1, 2 ybtAEPQSTUX	19	K19
CBWI01.1.fsa_nt	427	R, FII(K), FIB(K)	SHV-123, armB	ST11	p1220-CTXM	mrkABCDFHIJ, fyuA, irp1, 2, ybtAEPQSTUX	75	/
LYPT01.1.fsa_nt	104	R, FII(K), ColRNAI	SHV-158	ST11	+-p1220-CTXM	mrkAFIJ	104	/
NPGY01.1.fsa_nt[a]	208	R, FII(K), N	SHV-126	ST1582	KP048	mrkABD	161	/
LJOI01.1.fsa_nt	77	L/M, FII(K), R, FIB(K), N	SHV-126, NDM-1	ST14	pNDM-OM	mrkABCDFIJ, kfuABC	2	K2
FLVD01.1.fsa_nt	35	FII(K), FIA(pBK30683), FIB	SHV-1	ST199	pKp-Gop-414-4[b]	mrkABCFHIJ	411	/
LYPV01.1.fsa_nt	132	HI2, HI2A, FIB(K), FII(K),	SHV-11	ST517	p505108-MDR	mrkADFI	Related to 333	/
NOKM01.1.fsa_nt	114	pO111, FII(K), FIB(Mar), Col(BS512), X3, Col(MG828)	SHV-28, armA, NDM-5, mcr-1	ST15	UN	mrkABCDFIJ, kfuABC	29	K41
NQAU01.1.fsa_nt	83	FIB(pKPHS1)	SHV-11	ST37	UN	mrkABCDFHIJ	96	K38
BDLG01.1.fsa_nt	160	FIB(Mar), ColpVC, HI1B, R	SHV-27	ST485	UN	mrkADFHIJK	100	K10
NQEJ01.1.fsa_nt	75	HI1B, R	SHV-27	ST719	UN	mrkAFIJ	104	/
LYPW01.1.fsa_nt	233	FIB(K), HI2, FII(pBK30683), R, FII(K)	SHV-11	ST354	UN	mrkABHI	192	/
FLVT01.1.fsa_nt	48	FIB(pKPHS1), HI1B, FII(K)	SHV-27	ST234	UN	mrkABDFIJ	162	K35
NBXV01.1.fsa_nt	85	FIB(K), X3, R, FII(K)	SHV-12, NDM-1	ST1	UN	mrkABCDFIJ	45	K45
AQOC01.1.fsa_nt	183	FII, FIA(HI1), FII(K)	SIM-1, OKP-B-8	ST421	UN	mrkABCDFHIJ	44	/

UN unknown

[a] *Klebsiella variicola*

[b] Plasmid pKp-Gop-414-4 (NZ_CP018341.1)

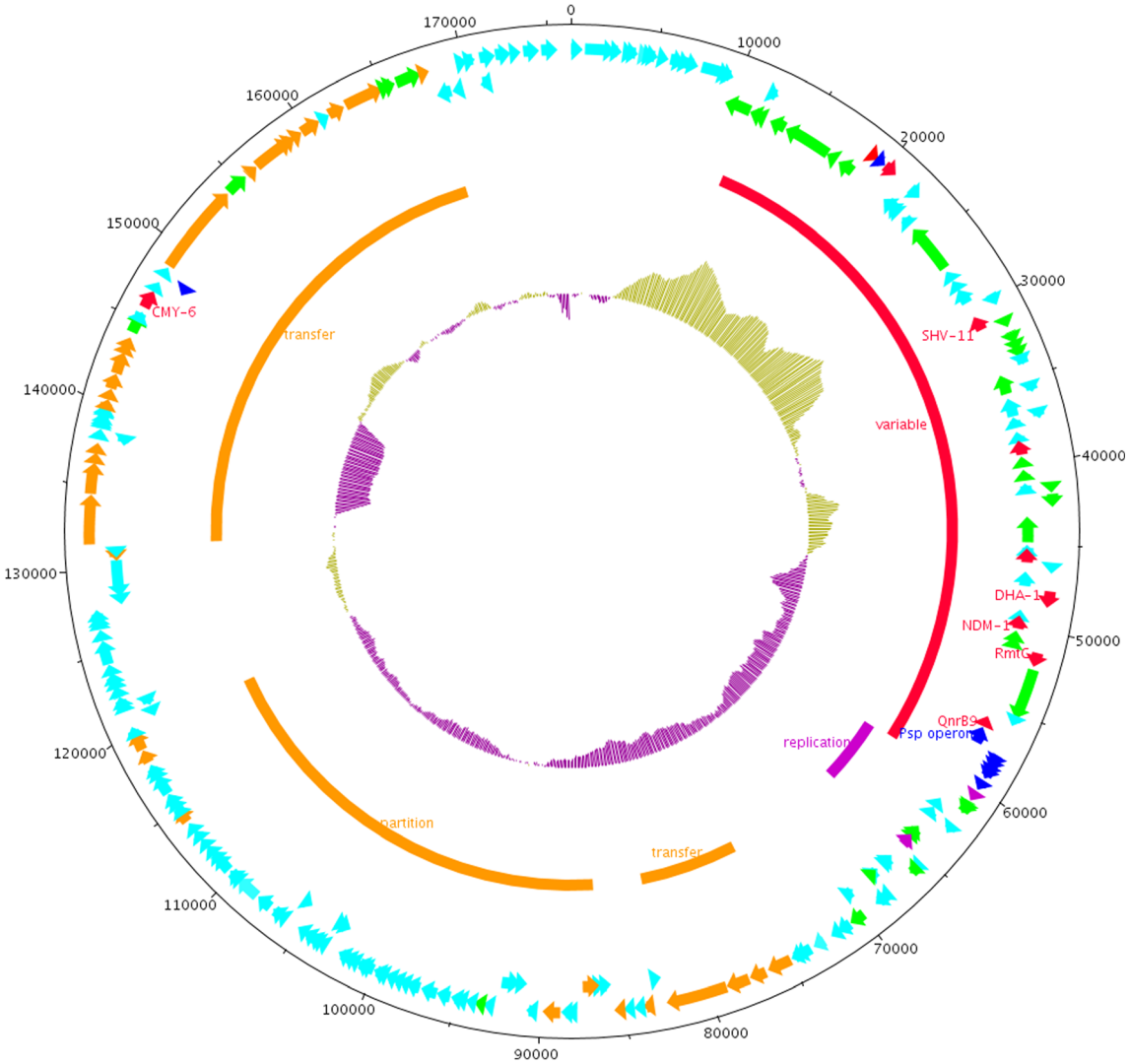

Fig. 1 An overview of the tig00000169_pilon plasmid (Accession: NZ_CP021952.1). The open reading frames were annotated in the second circle with arrows representing the direction of transcription. Mobile elements are indicated in green. Antibiotic resistance genes are indicated in red. The third circle indicates the functional sequence blocks. The G + C plot is indicated in the inner circle. The figure was made with DNAplotter [51]

between genes of the *sap* and *psp* operons; for pHS7, partial IS26 was located between *aphA1* and partial *sapB*.

In pKP048, IS26 was located at the 5′ and 3′ ends [25]. In Gram negative, most class I transposon are bounded by two copies of IS26 [55]. ISEc28, *armA*, ISEc29, and the *mel* and *mph* genes were found upstream of ISCR1. A *qacEΔ1sul1* segment was detected downstream of ISCR1 followed by the *M. morganii* region with *ampR* and bla_{DHA-1}, the *psp* operon, and *qnrB4*. Plasmid pB1025-1 shares the same genetic structure but without the *qnrB4* gene.

Isolates not carrying ISCR1 but IS26

In pTN60013, IS26 was detected at the 5′ and 3′ ends [15]. A partial *qacEΔ1sul1* region was found downstream of *ampR* and the *psp* operon upstream of the *M. morganii* region. In

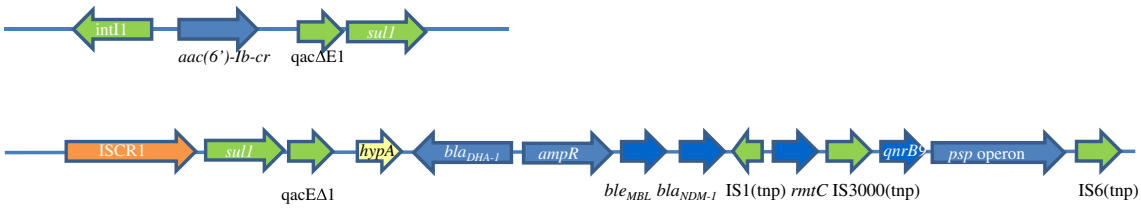

Fig. 2 Genetic organization of plasmid tig00000169_pilon

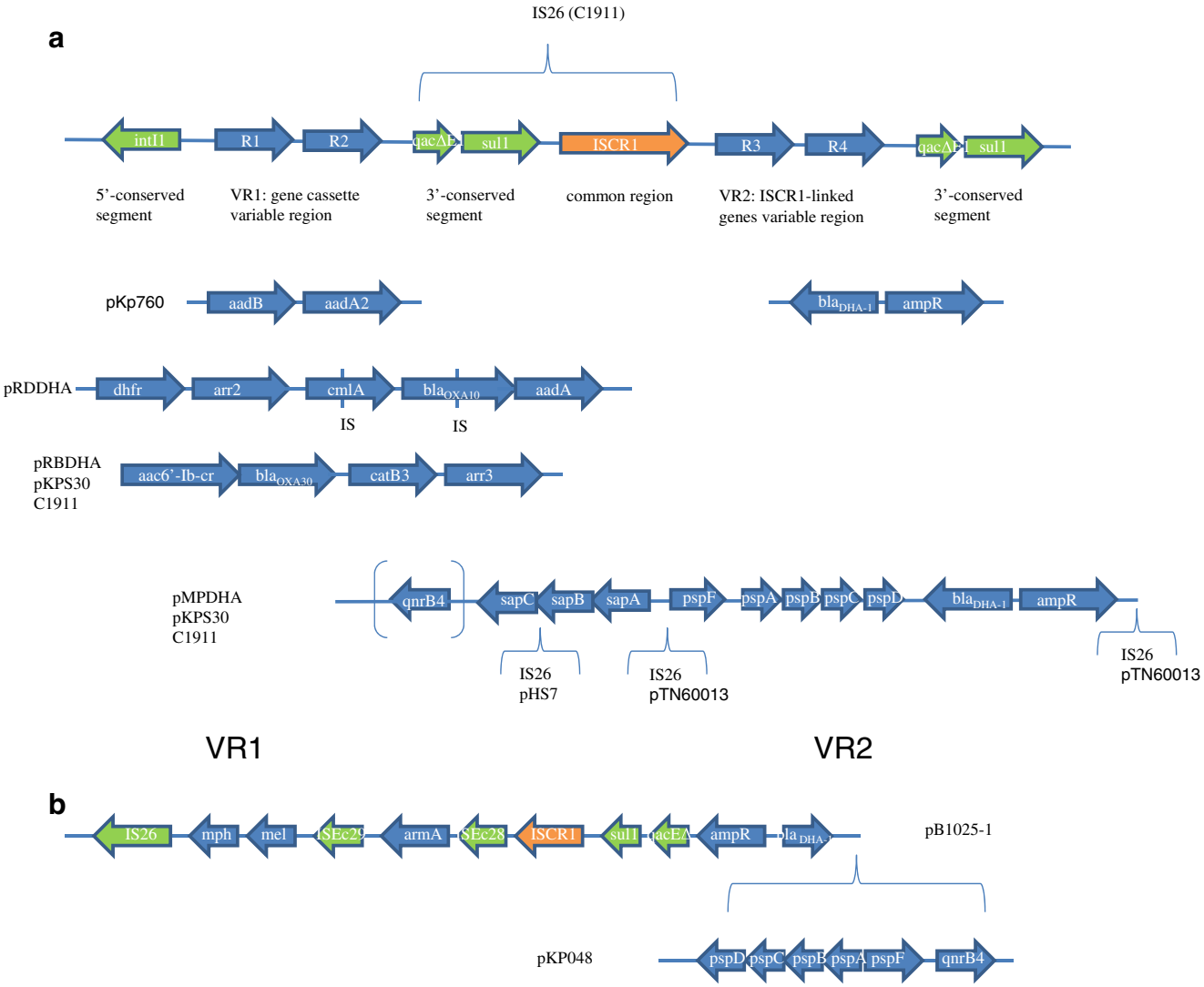

Fig. 3 Genetic context of ISCR1 element found in association with bla_{DHA-1} and *ampR* genes. Typical (**a**) and variant (**b**)

C1911, the integron was very close to the integron of pRBDHA but the *qacEΔ1sul1* region, the ISCR1 element, and the *sap* operon were missing, to the benefit of IS26 and *qnrB4*. IS26 moves via a replicative mechanism that deletes sequences immediately adjacent [55]. In pENVA, ISCR1 was also missing and IS26 was detected between the first *qacEΔ1sul1* region and *qnrB4* [41].

Conclusion

The first plasmid-mediated DHA-1 β-lactamase was isolated from a strain of *Salmonella* spp. in Saudi Arabia (Western Asia) in 1992 [4]. The following plasmids were reported in China and Korea (East Asia), then in the USA and finally in London, France, Belgium, and Spain (Europe). In the 1990s, the cases were sporadic, localized, and could be counted on the fingers of one hand. In the 2000s, the cases were worldwide and counted in tens. The strains were mainly isolated from human beings but also from pets (dogs, cats), sheep, and the environment (wastewater, river water). The first plasmid was isolated from a strain of *Salmonella* sp. but *Klebsiella* sp. strains, in particular *K. pneumoniae*, are its favorite host. The DHA-1 plasmids carried several antibiotic resistance genes and the strains could be considered as multi-resistant (MDR). The DHA-1 plasmid is rarely the only one in the strain and can be associated with one to six other Inc groups. The number of antibiotic resistance genes that could be present under an integron element in a strain is impressive and concerns all the antibiotic families. The genes can be located on the same region of the plasmid as that of the bla_{DHA-1} gene or on different regions or other plasmids. The increasing presence of carbapenemase enzymes in these strains is worrying and gives the feeling that these plasmids could harbor any kind of antibiotic resistance genes. Twenty-one complete DHA-1 plasmids have been sequenced: 13 from *K. pneumoniae*, 2 from *E. coli*, 3 from *C. freundii*, 1

from *Raoultella ornithinolytica*, 1 from *Cronobacter sakazakii*, and 1 from *Vibrio cholerae*, the only non-*Enterobacteriaceae*. Their size is variable and some of them are not self-transferable by conjugation. Seven Inc groups were found: R, A/C2, L/M, FII(k), HIB, FIB, and H12 sometimes with the presence of two hybrids. However, many plasmid-mediated DHA-1 β-lactamases remain unknown. Twenty-four STs have been listed, with ST11 and ST37 being predominant. ST11 is the most frequent clone of *K. pneumoniae* and is related to MDR strains [42]. The virulence of these strains has been little studied. They are mainly of classical pathogenesis and generally not hypervirulent [56]. DHA-1-producing *K. pneumoniae* strains are mainly responsible for nosocomial infections that affect people with weak health. The higher mortality observed with these infections was due to delay in initiating adequate antibiotic treatment [57, 58]. MDR and hypervirulent strains were usually non-overlapping [59] but these very diffusible plasmids can be found in a hypervirulent strain under antibiotic pressure, as seen for one of the strains from the Patric database. Some cases have already been reported [60] but it is to be hoped that the fitness cost of such an event is too high for the bacteria to be able to diffuse efficiently.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Jacoby GA (2009) AmpC beta-lactamases. Clin Microbiol Rev 22: 161–182, Table of Contents. https://doi.org/10.1128/CMR.00036-08
2. Papanicolaou GA, Medeiros AA, Jacoby GA (1990) Novel plasmid-mediated beta-lactamase (MIR-1) conferring resistance to oxyimino- and alpha-methoxy beta-lactams in clinical isolates of *Klebsiella pneumoniae*. Antimicrob Agents Chemother 34:2200–2209
3. Philippon A, Arlet G, Jacoby GA (2002) Plasmid-determined AmpC-type beta-lactamases. Antimicrob Agents Chemother 46:1–11
4. Gaillot O, Clément C, Simonet M, Philippon A (1997) Novel transferable beta-lactam resistance with cephalosporinase characteristics in *Salmonella* enteritidis. J Antimicrob Chemother 39:85–87
5. Ambler RP (1980) The structure of beta-lactamases. Philos Trans R Soc Lond Ser B Biol Sci 289:321–331
6. Bush K, Jacoby GA, Medeiros AA (1995) A functional classification scheme for beta-lactamases and its correlation with molecular structure. Antimicrob Agents Chemother 39:1211–1233
7. Nourrisson C, Tan RN, Hennequin C, Gibold L, Bonnet R, Robin F (2015) The MAST® D68C test: an interesting tool for detecting extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae*. Eur J Clin Microbiol Infect Dis Off Publ Eur Soc Clin Microbiol 34:975–983. https://doi.org/10.1007/s10096-014-2305-6
8. Maraskolhe DL, Deotale VS, Mendiratta DK, Narang P (2014) Comparision of three laboratory tests for detection of AmpC β lactamases in *Klebsiella* species and *E. coli*. J Clin Diagn Res JCDR 8:DC05–DC08. https://doi.org/10.7860/JCDR/2014/8256.4432
9. Reuland EA, Hays JP, de Jongh DMC, Abdelrehim E, Willemsen I, Kluytmans JAJW et al (2014) Detection and occurrence of plasmid-mediated AmpC in highly resistant gram-negative rods. PLoS One 9:e91396. https://doi.org/10.1371/journal.pone.0091396
10. Edquist P, Ringman M, Liljequist BO, Wisell KT, Giske CG (2013) Phenotypic detection of plasmid-acquired AmpC in *Escherichia coli*—evaluation of screening criteria and performance of two commercial methods for the phenotypic confirmation of AmpC production. Eur J Clin Microbiol Infect Dis Off Publ Eur Soc Clin Microbiol 32:1205–1210. https://doi.org/10.1007/s10096-013-1869-x
11. Pérez-Pérez FJ, Hanson ND (2002) Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. J Clin Microbiol 40:2153–2162
12. Alvarez M, Tran JH, Chow N, Jacoby GA (2004) Epidemiology of conjugative plasmid-mediated AmpC beta-lactamases in the United States. Antimicrob Agents Chemother 48:533–537
13. Moland ES, Black JA, Ourada J, Reisbig MD, Hanson ND, Thomson KS (2002) Occurrence of newer beta-lactamases in *Klebsiella pneumoniae* isolates from 24 U.S. hospitals. Antimicrob Agents Chemother 46:3837–3842
14. Liebana E, Batchelor M, Clifton-Hadley FA, Davies RH, Hopkins KL, Threlfall EJ (2004) First report of *Salmonella* isolates with the DHA-1 Amp. beta-lactamase in the United Kingdom. Antimicrob Agents Chemother 48:4492. https://doi.org/10.1128/AAC.48.11.4492.2004
15. Verdet C, Benzerara Y, Gautier V, Adam O, Ould-Hocine Z, Arlet G (2006) Emergence of DHA-1-producing *Klebsiella spp*. in the Parisian region: genetic organization of the ampC and ampR genes originating from Morganella morganii. Antimicrob Agents Chemother 50:607–617. https://doi.org/10.1128/AAC.50.2.607-617.2006
16. Pai H, Kang C-I, Byeon J-H, Lee K-D, Park WB, Kim H-B et al (2004) Epidemiology and clinical features of bloodstream infections caused by AmpC-type-beta-lactamase-producing *Klebsiella pneumoniae*. Antimicrob Agents Chemother 48:3720–3728. https://doi.org/10.1128/AAC.48.10.3720-3728.2004
17. Pai H, Seo M-R, Choi TY (2007) Association of QnrB determinants and production of extended-spectrum beta-lactamases or plasmid-mediated AmpC beta-lactamases in clinical isolates of *Klebsiella pneumoniae*. Antimicrob Agents Chemother 51:366–368. https://doi.org/10.1128/AAC.00841-06
18. Yan J-J, Ko W-C, Jung Y-C, Chuang C-L, Wu J-J (2002) Emergence of *Klebsiella pneumoniae* isolates producing inducible DHA-1 beta-lactamase in a university hospital in Taiwan. J Clin Microbiol 40:3121–3126
19. Kim J-Y, Park Y-J, Lee S-O, Song W, Jeong SH, Yoo YA et al (2004) Case report: bacteremia due to *Salmonella enterica* serotype Montevideo producing plasmid-mediated AmpC beta-lactamase (DHA-1). Ann Clin Lab Sci 34:214–217
20. Yan J-J, Ko W-C, Wu H-M, Tsai S-H, Chuang C-L, Wu J-J (2004) Complexity of *Klebsiella pneumoniae* isolates resistant to both cephamycins and extended-spectrum cephalosporins at a teaching hospital in Taiwan. J Clin Microbiol 42:5337–5340. https://doi.org/10.1128/JCM.42.11.5337-5340.2004
21. Roh KH, Uh Y, Kim J-S, Kim H-S, Shin DH, Song W (2008) First outbreak of multidrug-resistant *Klebsiella pneumoniae* producing both SHV-12-type extended-spectrum beta-lactamase and DHA-1-type AmpC beta-lactamase at a Korean hospital. Yonsei Med J 49: 53–57. https://doi.org/10.3349/ymj.2008.49.1.53
22. Tamang MD, Seol SY, Oh J-Y, Kang HY, Lee JC, Lee YC et al (2008) Plasmid-mediated quinolone resistance determinants *qnrA*, *qnrB*, and *qnrS* among clinical isolates of *Enterobacteriaceae* in a Korean hospital. Antimicrob Agents Chemother 52:4159–4162. https://doi.org/10.1128/AAC.01633-07
23. Hu F, Xu X, Zhu D, Wang M (2008) Coexistence of qnrB4 and qnrS1 in a clinical strain of *Klebsiella pneumoniae*. Acta

Pharmacol Sin 29:320–324. https://doi.org/10.1111/j.1745-7254.2008.00757.x

24. Lee C-H, Liu J-W, Li C-C, Chien C-C, Tang Y-F, Su L-H (2011) Spread of ISCR1 elements containing *bla DHA-1* and multiple antimicrobial resistance genes leading to increase of flomoxef resistance in extended-spectrum-beta-lactamase-producing *Klebsiella pneumoniae*. Antimicrob Agents Chemother 55:4058–4063. https://doi.org/10.1128/AAC.00259-11
25. Jiang Y, Yu D, Wei Z, Shen P, Zhou Z, Yu Y (2010) Complete nucleotide sequence of *Klebsiella pneumoniae* multidrug resistance plasmid pKP048, carrying bla_{KPC-2}, bla_{DHA-1}, *qnrB4*, and *armA*. Antimicrob Agents Chemother 54:3967–3969. https://doi.org/10.1128/AAC.00137-10
26. Zhang D, Yin Z, Zhao Y, Feng J, Jiang X, Zhan Z et al (2017) p1220-CTXM, a pKP048-related IncFIIK plasmid carrying $bla_{CTX-M-14}$ and *qnrB4*. Future Microbiol 12:1035–1043. https://doi.org/10.2217/fmb-2017-0026
27. Vanwynsberghe T, Verhamme K, Raymaekers M, Cartuyvels R, Boel A, De Beenhouwer H (2007) Outbreak of *Klebsiella pneumoniae* strain harbouring an AmpC (DHA-1) and a bla_{SHV-11} in a Belgian hospital, August–December 2006. Eur Surveill Bull Eur Sur Mal Transm Eur Commun Dis Bull 12:E070201.3
28. Mata C, Miró E, Toleman M, Rivera MA, Walsh TR, Navarro F (2011) Association of *bla(DHA-1)* and *qnrB* genes carried by broad-host-range plasmids among isolates of *Enterobacteriaceae* at a Spanish hospital. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis 17:1514–1517. https://doi.org/10.1111/j.1469-0691.2011.03539.x
29. Diestra K, Miró E, Martí C, Navarro D, Cuquet J, Coll P et al (2011) Multiclonal epidemic of *Klebsiella pneumoniae* isolates producing DHA-1 in a Spanish hospital. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis 17:1032–1036. https://doi.org/10.1111/j.1469-0691.2010.03319.x
30. Yu F, Chen C, Chen Q, Yu X, Ding B, Yang L et al (2012) Identification of transferable DHA-1 type AmpC β-lactamases and two mutations in quinolone resistance-determining regions of *Salmonella enterica* serovar Thompson. J Med Microbiol 61:460–462. https://doi.org/10.1099/jmm.0.040691-0
31. Yim G, Kwong W, Davies J, Miao V (2013) Complex integrons containing qnrB4-ampC (*bla(DHA-1)*) in plasmids of multidrug-resistant *Citrobacter freundii* from wastewater. Can J Microbiol 59:110–116. https://doi.org/10.1139/cjm-2012-0576
32. Pérez-Moreno MO, Estepa V, Sáenz Y, Cortell-Ortolá M, Fort-Gallifa I, Ruiz J et al (2012) Intrahospitalary dissemination of *Klebsiella pneumoniae* carrying *bla(DHA-1)* and *qnrB4* genes within a novel complex class 1 integron. Diagn Microbiol Infect Dis 73:210–211. https://doi.org/10.1016/j.diagmicrobio.2012.02.011
33. Guo Q, Wang P, Ma Y, Yang Y, Ye X, Wang M (2012) Co-production of SFO-1 and DHA-1 β-lactamases and 16S rRNA methylase ArmA in clinical isolates of *Klebsiella pneumoniae*. J Antimicrob Chemother 67:2361–2366. https://doi.org/10.1093/jac/dks244
34. Compain F, Frangeul L, Drieux L, Verdet C, Brisse S, Arlet G et al (2014) Complete nucleotide sequence of two multidrug-resistant IncR plasmids from *Klebsiella pneumoniae*. Antimicrob Agents Chemother 58:4207–4210. https://doi.org/10.1128/AAC.02773-13
35. Tobes R, Codoñer FM, López-Camacho E, Salanueva IJ, Manrique M, Brozynska M et al (2013) Genome sequence of *Klebsiella pneumoniae* KpQ3, a DHA-1 β-lactamase-producing nosocomial isolate. Genome Announc 1. https://doi.org/10.1128/genomeA.00167-12
36. Compain F, Decré D, Fulgencio J-P, Berraho S, Arlet G, Verdet C (2014) Molecular characterization of DHA-1-producing *Klebsiella pneumoniae* isolates collected during a 4-year period in an intensive care unit. Diagn Microbiol Infect Dis 80:159–161. https://doi.org/10.1016/j.diagmicrobio.2014.06.009
37. Hidalgo L, Gutierrez B, Ovejero CM, Carrilero L, Matrat S, Saba CKS et al (2013) *Klebsiella pneumoniae* sequence type 11 from companion animals bearing ArmA methyltransferase, DHA-1 β-lactamase, and QnrB4. Antimicrob Agents Chemother 57:4532–4534. https://doi.org/10.1128/AAC.00491-13
38. Ho PL, Lo WU, Yeung MK, Lin CH, Chow KH, Ang I et al (2011) Complete sequencing of pNDM-HK encoding NDM-1 carbapenemase from a multidrug-resistant *Escherichia coli* strain isolated in Hong Kong. PLoS One 6:e17989. https://doi.org/10.1371/journal.pone.0017989
39. Voulgari E, Poulou A, Dimitroulia E, Politi L, Ranellou K, Gennimata V et al (2015) Emergence of OXA-162 carbapenemase- and DHA-1 Amp. cephalosporinase-producing sequence type 11 *Klebsiella pneumoniae* causing community-onset infection in Greece. Antimicrob Agents Chemother 60:1862–1864. https://doi.org/10.1128/AAC.01514-15
40. Kuai S, Shao H, Huang L, Pei H, Lu Z, Wang W et al (2014) KPC-2 carbapenemase and DHA-1 Amp. determinants carried on the same plasmid in *Enterobacter aerogenes*. J Med Microbiol 63:367–370. https://doi.org/10.1099/jmm.0.054627-0
41. Schlüter A, Nordmann P, Bonnin RA, Millemann Y, Eikmeyer FG, Wibberg D et al (2014) IncH-type plasmid harboring $bla_{CTX-M-15}$, bla_{DHA-1}, and *qnrB4* genes recovered from animal isolates. Antimicrob Agents Chemother 58:3768–3773. https://doi.org/10.1128/AAC.02695-14
42. Kis Z, Tóth Á, Jánvári L, Damjanova I (2016) Countrywide dissemination of a DHA-1-type plasmid-mediated AmpC β-lactamase-producing *Klebsiella pneumoniae* ST11 international high-risk clone in Hungary, 2009-2013. J Med Microbiol 65:1020–1027. https://doi.org/10.1099/jmm.0.000302
43. Taniguchi Y, Maeyama Y, Ohsaki Y, Hayashi W, Osaka S, Koide S et al (2017) Co-resistance to colistin and tigecycline by disrupting *mgrB* and *ramR* with IS insertions in a canine *Klebsiella pneumoniae* ST37 isolate producing SHV-12, DHA-1 and FosA3. Int J Antimicrob Agents 50:697–698. https://doi.org/10.1016/j.ijantimicag.2017.09.011
44. Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L et al (2014) *In silico* detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother 58:3895–3903. https://doi.org/10.1128/AAC.02412-14
45. McArthur AG, Waglechner N, Nizam F, Yan A, Azad MA, Baylay AJ et al (2013) The comprehensive antibiotic resistance database. Antimicrob Agents Chemother 57:3348–3357. https://doi.org/10.1128/AAC.00419-13
46. Wattam AR, Davis JJ, Assaf R, Boisvert S, Brettin T, Bun C et al (2017) Improvements to PATRIC, the all-bacterial bioinformatics database and analysis resource center. Nucleic Acids Res 45:D535–D542. https://doi.org/10.1093/nar/gkw1017
47. Darling AE, Mau B, Perna NT (2010) progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One 5:e11147. https://doi.org/10.1371/journal.pone.0011147
48. Lawlor MS, O'connor C, Miller VL (2007) Yersiniabactin is a virulence factor for *Klebsiella pneumoniae* during pulmonary infection. Infect Immun 75:1463–1472. https://doi.org/10.1128/IAI.00372-06
49. Nassif X, Sansonetti PJ (1986) Correlation of the virulence of *Klebsiella pneumoniae* K1 and K2 with the presence of a plasmid encoding aerobactin. Infect Immun 54:603–608
50. Cheng HY, Chen YS, Wu CY, Chang HY, Lai YC, Peng HL (2010) RmpA regulation of capsular polysaccharide biosynthesis in *Klebsiella pneumoniae* CG43. J Bacteriol 192:3144–3158. https://doi.org/10.1128/JB.00031-10
51. Carver T, Thomson N, Bleasby A, Berriman M, Parkhill J (2009) DNAPlotter: circular and linear interactive genome visualization. Bioinforma Oxf Engl 25(1):119–120. https://doi.org/10.1093/bioinformatics/btn578

52. Chérif T, Saidani M, Decré D, Boutiba-Ben Boubaker I, Arlet G (2015) Cooccurrence of multiple AmpC β-lactamases in *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* in Tunisia. Antimicrob Agents Chemother 60:44–51. https://doi.org/10.1128/AAC.00828-15
53. Toleman MA, Bennett PM, Walsh TR (2006) ISCR elements: novel gene-capturing systems of the 21st century? Microbiol Mol Biol Rev MMBR 70:296–316. https://doi.org/10.1128/MMBR.00048-05
54. Cheng C, Sun J, Zheng F, Lu W, Yang Q, Rui Y (2016) New structures simultaneously harboring class 1 integron and ISCR1-linked resistance genes in multidrug-resistant Gram-negative bacteria. BMC Microbiol 16:71. https://doi.org/10.1186/s12866-016-0683-x
55. Harmer CJ, Hall RM (2016) IS26-mediated formation of transposons carrying antibiotic resistance genes. mSphere 1. https://doi.org/10.1128/mSphere.00038-16
56. Hennequin C, Robin F, Cabrolier N, Bonnet R, Forestier C (2012) Characterization of a DHA-1-producing *Klebsiella pneumoniae* strain involved in an outbreak and role of the AmpR regulator in virulence. Antimicrob Agents Chemother 56:288–294. https://doi.org/10.1128/AAC.00164-11
57. Luk S, Wong W-K, Ho AY-M, Yu KC-H, To W-K, Ng T-K (2016) Clinical features and molecular epidemiology of plasmid-mediated DHA-type AmpC β-lactamase-producing *Klebsiella pneumoniae* blood culture isolates, Hong Kong. J Glob Antimicrob Resist 7:37–42. https://doi.org/10.1016/j.jgar.2016.06.006
58. Ku Y-H, Chuang Y-C, Chen C-C, Lee M-F, Yang Y-C, Tang H-J et al (2017) *Klebsiella pneumoniae* isolates from meningitis: epidemiology, virulence and antibiotic resistance. Sci Rep 7:6634. https://doi.org/10.1038/s41598-017-06878-6
59. Bialek-Davenet S, Criscuolo A, Ailloud F, Passet V, Jones L, Delannoy-Vieillard A-S et al (2014) Genomic definition of hypervirulent and multidrug-resistant *Klebsiella pneumoniae* clonal groups. Emerg Infect Dis 20:1812–1820. https://doi.org/10.3201/eid2011.140206
60. Cheong HS, Chung DR, Lee C, Kim SH, Kang C-I, Peck KR et al (2016) Emergence of serotype K1 *Klebsiella pneumoniae* ST23 strains co-producing the plasmid-mediated AmpC beta-lactamase DHA-1 and an extended-spectrum beta-lactamase in Korea. Antimicrob Resist Infect Control 5:50. https://doi.org/10.1186/s13756-016-0151-2