BRAZ J INFECT DIS. 2015;19(2):187–195

The Brazilian Journal of
INFECTIOUS DISEASES

www.elsevier.com/locate/bjid

Original article

Molecular characterization and epidemiology of cefoxitin resistance among *Enterobacteriaceae* lacking inducible chromosomal *ampC genes* from hospitalized and non-hospitalized patients in Algeria: description of new sequence type in *Klebsiella pneumoniae* isolates

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ARTICLE INFO

Article history:
Received 11 August 2014
Accepted 17 December 2014
Available online 28 January 2015

Keywords:
Enterobacteriaceae
AmpC β-lactamases
Genotyping
Algeria

ABSTRACT

In this study, 922 consecutive non-duplicate clinical isolates of *Enterobacteriaceae* obtained from hospitalized and non-hospitalized patients at Bejaia, Algeria were analyzed for AmpC-type β-lactamases production. The *ampC* genes and their genetic environment were characterized using polymerase chain reaction (PCR) and sequencing. Plasmid incompatibility groups were determined by using PCR-based replicon typing. Phylogenetic grouping and multilocus sequence typing were determined for molecular typing of the plasmid-mediated AmpC (pAmpC) isolates.

Of the isolates, 15 (1.6%) were identified as AmpC producers including 14 CMY-4-producing isolates and one DHA-1-producing *Klebsiella pneumoniae*. All AmpC-producing isolates co-expressed the broad-spectrum TEM-1 β-lactamase and three of them co-produced CTX-M and/or SHV-12 ESBL. Phylogenetic grouping and virulence genotyping of the *E. coli* isolates revealed that most of them belonged to groups D and B1. Multilocus sequence typing analysis of *K. pneumoniae* isolates identified four different sequence types (STs) with two new sequences: ST1617 and ST1618. Plasmid replicon typing indicates that $bla_{\text{CMY-4}}$ gene was located on broad host range A/C plasmid, while LVPK replicon was associated with $bla_{\text{DHA-1}}$. All isolates carrying $bla_{\text{CMY-4}}$ displayed the transposon-like structures ISEcp1/ΔISEcp1-blaCMY-blc-sugE.

Our study showed that CMY-4 was the main pAmpC in the *Enterobacteriaceae* isolates in Algeria.

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http://dx.doi.org/10.1016/j.bjid.2014.12.001
1413-8670/

Introduction

Infection with resistant organisms is a major public health issue. *Enterobacteriaceae* are important causes of both community-acquired and healthcare-associated infections in adults and children, and production of β-lactamases is of greater concern.[1] Plasmid-mediated AmpC (pAmpC) β-lactamases have emerged and are being reported worldwide with varying prevalence rates.[2] They have been mainly detected in *Escherichia coli*, *Klebsiella* spp., *Salmonella* spp., and *Proteus mirabilis*.[3] These enzymes confer resistance to penicillins, first and third generation cephalosporins, cephamycins, and monobactams such as aztreonam. They are poorly inhibited by the commercially available β-lactamase inhibitors such as clavulanic acid, but are inhibited by cloxacillin and phenylboronic acid. Treatment options are severely limited because pAmpC are often associated with other multiple resistance genes, such as those of resistance to quinolones as well as other β-lactamase genes.[3,4] The acquired *ampC* genes have emerged following mobilizations mediated by such elements as IS*26*, IS*Ecp1*, or ISCR*1*.[3] Thus, IS*Ecp1* has played an important role in mobilizing bla_{CMY-2}-like genes, since it is often found at their 5′ flanks.[5,6] It has been identified in plasmids of the Inc A/C and Inc I1 groups.[5–7] ISCR*1* elements have been found adjacent to a number of *ampC* genes, including bla_{DHA-1}, as well as plasmid-mediated quinolone resistance determinant *qnr*.[8] Generally, the bla_{DHA-1} gene has been mainly associated with Inc FII and Inc L/M plasmids.[9]

In Algeria, only few reports on plasmid-encoded AmpC (CMY-2 and DHA-1) in *Enterobacteriaceae* strains recovered from hospital settings were published.[10–12]

The aim of this study was to investigate the prevalence and molecular epidemiology of cefoxitin resistance among *Enterobacteriaceae* isolates recovered from hospitalized and non-hospitalized patients in Bejaia locality (Algeria). The association of pAmpC with extended-spectrum β-lactamase (ESBL) and plasmid-mediated quinolone resistance determinant was also studied.

Materials and methods

Bacterial strains

A total of 922 non-duplicate isolates (one per patient) of *Enterobacteriaceae* were collected from March 2005 to April 2010 from the Microbiology Laboratories of five hospitals and four private laboratories in Bejaia (Algeria). The isolates were recovered from various pathological specimens and were identified by the API 20E system (bioMérieux, Marcy l'Etoile, France) as follows: *E. coli* (*n* = 551); *Klebsiella pneumoniae* (*n* = 221); *P. mirabilis* (*n* = 125) and *Salmonella* sp. (*n* = 25).

E. coli J53Az^R was used as recipient strains for conjugation experiments. *E. coli* DH10B (Invitrogen) was used in transformation experiments and *E. coli* ATCC 25922 was used as a quality control strain for antimicrobial susceptibility testing.

Antimicrobial susceptibility testing

Antibiotic susceptibility was determined on Mueller Hinton agar by standard disk diffusion procedure as described by the European Committee on Antimicrobial Susceptibility Testing (2014),[13] for the following antibiotics: aztreonam, ticarcillin, piperacillin, amoxicillin–clavulanate, ticarcillin–clavulanate, cefoxitin, cefepime, piperacillin–tazobactam, cefuroxime, cefotaxime, ceftazidime, imipenem, tobramycin, amikacin, gentamicin, sulfonamide, trimethoprim, nalidixic acid, ciprofloxacin, norfloxacin, tetracycline, and chloramphenicol (BioRad, Marnes La Coquette, France). For tetracycline, the Antibiogram Committee of the French Society for Microbiology recommendations breakpoints were used (http://www.sfm-microbiologie.org).

Isolates showing a zone of inhibition diameter ≤20 mm with cefoxitin were selected for screening for *pampC* genes. ESBL production was detected by a double-disk synergy test (DDST) on Mueller Hinton supplemented with cloxacillin (200 mg/L).[14] Inducibility of the β-lactamase was determined by the double disk test. The cephalosporins used were cefotaxime, ceftazidime, and cefepime. Clavulanic acid (10 μg) and cefoxitin (30 μg) were used as inducing agents. The plates were examined after overnight incubation at 37 °C.[15]

Minimum inhibitory concentrations (MICs) of amoxicillin, amoxicillin/clavulanate, piperacillin/tazobactam, cefotaxime, ceftazidime, cefoxitin, imipenem, aztreonam, and cefepime were determined by Etest (AB bioMérieux, Marcy l'Etoile, France).

Molecular characterization of resistance determinants

Total DNA was extracted by using a QIAmp DNA Mini Kit (QIAGEN) according to the instructions of the manufacturer. A multiplex PCR covering the six families of *ampC* genes (CMY-2/BIL/LAT, CMY-1/MOX, DHA, FOX, ACC, ACT/MIR) was performed as previously described.[16] PCR-positive isolates were further tested using individual pairs of primers and then sequenced. pAmpC-producing isolates positive for the DDST were screened for bla_{CTX-M}, bla_{TEM} and bla_{SHV} by PCR as described previously.[17]

Screening of *qnrA*, *qnrB*, *qnrS*, *qnrC*, *qnrD* and *qepA* genes was carried out with a multiplex real-time PCR assay using SYBR Green I and Roche LightCycler1 as described previously.[18] Pyrosequencing method was used for the detection of *aac(6′)-Ib-cr* and *aac(6′)-Ib* genes.[19]

All PCR products were sequenced and the sequencing results were compared to reported sequences available in GenBank.

Transfer of resistance

Conjugation was performed on Mueller Hinton agar supplemented with sodium azide (100 mg/L) and cefotaxime (1 mg/L). Transconjugants growing on the selection plates were subjected to antimicrobials susceptibility, DDST and PCR analysis to confirm the presence of the AmpC phenotype.

Molecular typing

Possible genomic relatedness of strains was analyzed by RAPD using genomic DNA as previously described.[20] Multilocus sequence typing (MLST) was performed on the *K. pneumoniae* isolates using seven conserved housekeeping genes (*gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB* and *tonB*).[21] A detailed protocol of the MLST procedure, including allelic type and ST assignment methods, is available in MLST databases from the Pasteur Institute, Paris, France, at the website http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html.

Phylogenetic groups and virulence genotyping of E. coli

PCRs were performed to determine the phylogenetic groups (A, B1, B2, C, D, E, F, and clade I) of the *E. coli* isolates, using the newly revised Clermont method.[22] All isolates belonging to group B2 were analyzed by two multiplex PCR as described previously.[23] The presence of eight virulence factors found in ExPEC was investigated by PCR. These factors included *sfa/foc* (S and F1C fimbriae), *papG* alleles (G adhesin classes of P fimbriae), *papC* (C adhesin classes of P fimbriae), *hlyA* (alpha-haemolysin A), *cnf* (cytotoxic necrotizating factor 1), *fyuA* (genes of yersiniabactin), *iutA* (aerobactin receptor), and *ibeA* (invasion protein IbeA).

Plasmid replicon typing

Plasmids incompatibility (Inc) groups were determined using PCR-based replicon typing (PBRT).[24] Four multiplex PCR were used for the detection of A/C, T, FIIAs, W, N, FIB, L/M, I1-Iγ, X, HI2, FIA, and Y replicons. Replicons P, R, U, F, FIC, HI1, B/O and K were detected by simplex PCR.[24,25] Replicons FII1K, FII2K, NewXXX also named ZK, LVPK, and Amet were detected using PCR method described by D. Decré and G. Arlet.

Genetic organization of bla_{ampC} genes

For the analysis of genetic arrangement of the resistance genes, overlapping PCR amplification of internal regions of the transposon-like element that carried bla_{CMY-4} was performed based on known sequences.

Genetic structures surrounding the bla_{DHA-1} gene were studied by PCR mapping, cloning and sequencing method using a large variety of primers based on the previously reported structures.[8]

The nucleotide sequence and the deduced protein sequence were analyzed using the Basic Local Alignment Search Tool (BLAST) through the Internet (http://www.ncbi.nlm.nih.gov/BLAST/). Multiple sequence alignment of deduced peptide sequences was carried out with the Vector NTI program (Invitrogen).

Results

Bacterial isolates and antibiotic susceptibility

Among the 922 *Enterobacteriaceae* isolates, 15 isolates showed decreased susceptibility to cefoxitin: nine isolates of *E. coli* (9/551), five isolates (5/221) of *K. pneumoniae* and one isolate (1/125) of *P. mirabilis*. Ten isolates were recovered from urine. Six *E. coli* isolates and one *K. pneumoniae* isolate were from community patients (7/712), and the remaining eight isolates were collected from hospitalized patients (8/210).

All isolates exhibited resistance to ticarcillin, piperacillin, ticarcillin–clavulanic acid, amoxicillin–clavulanic acid, cefuroxime, cefotaxime, ceftazidime, aztreonam, and cefoxitin. Isolates exhibited intermediate resistance to piperacillin–tazobactam (60%) and cefepime (40%). Resistance of the isolates to non-β-lactam antibiotics was high for sulfonamide (80%), tobramycin (71.4%), gentamicin (71.4%), tetracycline (71.4%), chloramphenicol (64.3%) and trimethoprim (57.1%), and low for nalidixic acid (28.6%) and amikacin (6.6%). The isolates remain susceptible to imipenem and fluoroquinolones. The MICs ranges are listed in Table 1.

The ESBL phenotypic screening by double disk diffusion synergy test showed that one isolate of *K. pneumoniae* and two isolates of *E. coli* were ESBL producers (Table 1).

Inducibility of β-lactamases was recognized by the disk antagonism test, which demonstrated blunting of the cephalosporin disks adjacent to the cefoxitin and clavulanic acid disks in only one isolate of *K. pneumoniae* 413. This phenotype suggested the presence of an inducible AmpC-type β-lactamase.

Genotypic analysis of antibiotic resistance genes

By multiplex PCR, we obtained amplicons in 15 isolates: nine *E. coli* isolates, five *K. pneumoniae* isolates, and one *P. mirabilis* isolate (Table 1).

PCR and sequencing analysis revealed the presence of bla_{CMY-4} in all isolates except one isolate of *K. pneumonia*, which produced bla_{DHA-1}.

In addition, two *E. coli* (CMY-4) co-produced CTX-M-15 ESBLs and one isolate of *K. pneumoniae* (DHA-1) co-produced CTX-M-3 and SHV-12 ESBL. All isolates carried bla_{TEM-1}.

PCR amplification of PMQR yielded amplification in one *K. pneumoniae* isolate only. This strain expressed both the *qnrB4*, *aac(6′)-Ib*, bla_{DHA-1}, $bla_{CTX-M-3}$, bla_{SHV-12} and bla_{TEM-1} genes (Table 1).

No amplicons were obtained for *qnrA*, *qnrS*, *qnrD*, *qnrC* and *qepA* in all isolates.

Conjugation and replicon typing

By mating assay, the *ampC* genes were transferred from three of the five *K. pneumoniae*, five of the nine *E. coli* isolates and from the *P. mirabilis* isolate. Susceptibility results of the transconjugants are shown in Table 1.

PBRT of the plasmid Inc groups showed that the plasmids carrying bla_{CMY-4} belonged to the Inc A/C group and the plasmid carrying bla_{DHA-1} belonged to the group Inc LVPK (Table 1).

Molecular typing

RAPD-typing revealed the presence of diverse bacterial population and no predominant clone was identified in our collection.

Table 1 – Microbiological features of pAmpC producing *Enterobacteriaceae*.

Isolates and transconjugants	Date of isolation	Specimen	Hospital/ private laboratory	Ward	Plasmid-mediated bla_{AmpC}	Other β-lactamases genes	PMQRs genes	MICs (mg/L)									RAPD clone	MLST (*K. pneumoniae*)	Replicon typing
								AMX	AMC	TZP	CTX	CAZ	ATM	FEP	IMP	FOX			
K. pneumoniae 47	04/04/2007	Urine	AWH	Surgery	CMY-4	TEM-1	–	>256	48	>256	>32	>256	32	<04	0.38	64	K.A.	17	Inc A/C, LVPK
TC47					CMY-4		–	>256	16	128	>32	128	32	<04	0.19	32			Inc A/C
K. pneumoniae 123	04/05/2005	Urine	AWH	Pediatrics	CMY-4	TEM-1	–	>256	32	>256	>32	>256	32	>64	0.38	64	K.B.	1617	Inc A/C, FIB, F, P
TC123					CMY-4		–	>256	16	16	12	12	<04	<04	0.25	32			Inc A/C
K. pneumoniae 613	05/04/2010	Feces	AZH	Medicine	CMY-4		–	>256	16	08	>32	192	32	<04	0.25	64	K.D.	1618	ND
K. pneumoniae 615	03/05/2010	Feces	AZH	Medicine	CMY-4		–	>256	16	08	>32	192	32	<04	0.25	64	K.D.	1618	ND
K. pneumoniae 413	21/04/2008	Feces	AZH	Surgery	DHA-1	TEM-1; CTX-M-3; SHV-12	QnrB4	>256	16	02	>32	48	32	16	0.19	16	K.C.	834	Inc L/M, LVPK, HI2
TC413					DHA-1	TEM-1	QnrB4, acc(6)-Ib	>256	02	0.38	0.19	03	<04	<04	0.032	16			Inc LVPK
E. coli 234	15/04/2007	Urine	AZH	Surgery	CMY-4	TEM-1; CTX-M-15	–	>256	32	>256	>32	>256	32	>64	0.5	64	E.A.		Inc A/C, F, P, FII2K
TC234					CMY-4		–	>256	16	12	>32	96	32	<04	0.25	64			Inc A/C
E. coli 412	22/04/2008	Feces	AZH	Pediatrics	CMY-4	TEM-1	–	>256	32	>256	>32	>256	32	<04	0.5	32	E.B.		Inc A/C, FIA, A/C, FIB, F, B/O, U
TC412					CMY-4		–	>256	16	>256	>32	48	32	<04	0.25	32			Inc A/C,
E. coli 535	14/04/2009	Urine	LPL	Community	CMY-4	TEM-1	–	>256	16	16	>32	>256	32	<04	0.25	64	E.C.		Inc A/C, FIB, HI1
E. coli 538	14/02/2009	Urine	LPL	Community	CMY-4	TEM-1	–	>256	16	08	>32	128	32	<04	0.19	64	E.D.		Inc A/C, FIB, FIA, FII 1K, U
TC538			LPL		CMY-4		–	>256	16	04	>32	64	32	<04	0.19	32			Inc A/C
E. coli 539	05/04/2009	Urine	LPL	Community	CMY-4	TEM-1	–	>256	16	16	>32	>256	32	<04	0.25	64	E.C.		Inc A/C, FIB, HI1
E. coli 545	24/02/2009	Urine	LPL	Community	CMY-4	TEM-1	–	>256	16	08	>32	128	32	<04	0.19	64	E.D.		Inc A/C, FIB, FIA, FII 1K, U
E. coli 560	01/12/2009	Urine	LPL	Community	CMY-4	TEM-1	–	>256	16	08	>32	128	32	<04	0.19	64	E.D.		Inc A/C, FIB, FIA, FII 1K, U
E. coli 606	21/04/2010	Urine	DPL	Community	CMY-4	TEM-1	–	>256	16	04	>32	48	08	<04	0.19	32	E.E.		ND

E. coli 611	28/05/2010	Feces	AZH	Surgery	CMY-4	TEM-1, CTX-M-15	–	>256	16	04	>32	64	32	16	0.25	64	E.F.	Inc FIA, I1, F, U, FII 1K
TC611					CMY-4		–	>256	16	02	>32	08	32	<04	0.25	32		Inc A/C
P. mirabilis 128	07/04/2005	Urine	AZH	Surgery	CMY-4	TEM-1	–	>256	48	12	>32	48	<04	<04	0.25	32	–	Inc A/C
TC128					CMY-4		–	>256	16	0.75	>32	24	<04	<04	0.25	32		Inc A/C

LPL, Lalaoui private laboratory; DPL, Djama private laboratory; AZH, Amizour hospital; AWH, Amriw hospital; TZP, piepracillin–tazobactam; AMX, amoxicillin; AMC, amoxicillin–clavulanic acid; FOX, cefoxitin; ATM, aztreonam; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; IMP, imipenem; ND, not determined.

MLST analysis of the five AmpC-producing *K. pneumoniae* identified four different STs, including ST17, ST834 and two new sequence types: ST1617 (*Kp* 123) and ST1618 (*Kp* 613 and 615) (Table 1). In ST1618, we described a new allele's *mdh* and *rpoB* designated respectively 145 and 108. The typing results generated by RAPD analysis among the isolates were compatible with those obtained by MLST.

E. coli phylogenetic groups and virulence factors

Of the nine *E. coli* isolates, three belonged to group D, three to group B1 (recovered from urine), two to group B2, and the last one to group F (Table 2).

Five isolates harbored genes encoding siderophores (*fyuA*, *iutA*).

The *E. coli* 412 isolate was assigned to the B2 sub-group VII and STc14. This strain was isolated from the feces of a hospitalized patient. This isolate contained a $bla_{\text{TEM-1}}$ gene and, a $bla_{\text{CMY-4}}$ gene, which was transferred with an Inc A/C plasmid. The following virulence genes (*papC*, *papG II*, *sfa*, *hlyA*, *cnf1*, *fyuA* and *iutA*) were detected in this isolate (Table 2).

By using allele-specific PCR method for detecting the main *E. coli* B2 STc, *E. coli* 611 isolate was unassigned; it did not give any PCR products except for the internal control.

Characterization of the genetic contexts of bla_{AmpC} *genes*

Analysis of the genetic structure of the $bla_{\text{CMY-4}}$ gene in our collection showed that it was located on a transposon-like DNA element consisting of a specific IS*Ecp1*/ΔIS*Ecp1*-$bla_{\text{CMY-4}}$-*blc-sugE* structure. This structure was similar to that found in plasmid pCC416 (GenBank AJ875405).

A region typical of a complex *sul1*-type integron, from the *int* gene to CR1 was amplified using PCR mapping and then sequenced. By cloning the region encompassing *ampC* and *ampR*, a recombinant plasmid (p413C) with an insert that conferred inducible resistance to ceftazidime was selected. The insert was found to contain $bla_{\text{DHA-1}}$ and the regulatory gene *ampR*, which was downstream of $bla_{\text{DHA-1}}$. This insert shared also part of pRBDHA's backbone carrying a complex integron (GenBank AJ971343). PCR and DNA sequencing results confirmed that the plasmid encoded at least three β-lactamase genes: $bla_{\text{TEM-1}}$, $bla_{\text{SHV-12}}$, and $bla_{\text{DHA-1}}$, and a plasmid mediated resistance to quinolone (QnrB4).

Discussion

pAmpC have been found worldwide but are less common than ESBLs.[3] They are emerging worldwide in various species of *Enterobacteriaceae* as a mechanism of acquired resistance to cefoxitin. In our study 1.6% ($n = 15$) of the screened *Enterobacteriaceae* isolates were cefoxitin-resistant and produced plasmid-mediated AmpC β-lactamases. Prevalence of pAmpC in Algeria is not known, due to the limited number of epidemiological surveys. In Algeria, Iabaden et al. reported a prevalence of plasmid mediated AmpC β-lactamases of 2.18%.[11] Mata et al. reported a significant increase in overall prevalence of *Enterobacteriaceae* carrying acquired AmpC in a Spanish hospital which was 0.43%, rising from 0.06% (1999) to 1.3% (2007).[26] A prevalence of 12.5% was reported by Mohamudha et al. in India.[27]

Our study demonstrated that pAmpC-producing *Enterobacteriaceae* might be the cause of nosocomial and community infections in Algeria. Of note, we found that 40% of the cases were recovered from non-hospitalized patients. Isolation of pAmpC-producing *Enterobacteriaceae* from community was reported by many authors.[28,29] Nursing homes and community-based sources of pAmpC-producers can pose a serious risk of transmission to hospitalized patients when infected or colonized patients are admitted. Gude et al. have found this resistance mechanism on isolates from community patients in a high rate, underscoring the need for close surveillance of these isolates.[30] Several studies reported the isolation of pAmpC-producing *Enterobacteriaceae* isolates from food products, such as retail chicken meat, retail meat, and cheese.[31–33] Thus, food chain might be a relevant vehicle for transmission of these enzymes in the community. They have also been detected in drinking water and river beaches.[34] These sources could contribute to the spread of global pAmpC-producers in addition to a possible transmission of mobile genetic elements carrying resistance genes among strains.[30]

In Algeria, CMY-2 and DHA-1 were previously reported by Messai et al. (2006)[10], Iabadene et al. (2009)[11] and Nedjai et al. (2012).[12] This is the first isolation of CMY-4 in clinical isolates

Table 2 – Distribution and combination patterns of virulence genes and phylogenetic groups detected in pAmpC-producing *E. coli*.

Strain	Adhesin			Toxin		Iron system		Invasin	Phylogenetic group
	papC	*papG II*	*sfa*	*hlyA*	*cnf1*	*fyuA*	*iutA*	*ibeA*	
234	–	–	–	–	–	–	–	–	F
412	+	+	+	+	+	+	+	–	B2
535	–	–	–	–	–	+	+	–	D
538	–	–	–	–	–	–	–	–	B1
539	–	–	–	–	–	+	+	–	D
545	–	–	–	–	–	–	–	–	B1
560	–	–	–	–	–	–	–	–	B1
606	–	–	–	–	–	+	+	–	D
611	–	–	–	–	–	+	+	–	B2

Groups F and B1 were not found in any selected virulence genes.

(nosocomial and community infections) in Algeria. Thus, the first strain (*K. pneumoniae* 123) was isolated in 2005 from a patient hospitalized at Bejaia hospital (Algeria). The predominance of CMY-4 was consistent with worldwide observations. DHA-1 has been mostly reported in Asia.[5,35]

In our study, *E. coli* isolates were mainly groups B2 and D strains which are commonly extra-intestinal pathogenic strains, while phylogenetic groups A and B1 strains, usually commensal, were less frequent.[36] CMY-2 production was reported in phylogenetic group D *E. coli* in humans and stray dogs.[5,37]

In the study of Mnif et al., the non-ST131-group B2 isolates, which were associated to CTX-M-15 ESBLs, had a higher frequency of several genes encoding key virulence factors such as adhesins *hra*, *sfa/foc*, *papC* and *papG II*, and the toxins *hylA* and *cnf1* than had the ST131 isolates.[38] In our study, a single isolate harbored several virulence genes *iutA*, *papC* and *sfa/foc* and belonged to phylogenetic group B2.

Our results showed that AmpC-producing *K. pneumoniae* isolates belonged to different sequence types. ST17 has been previously found in Cadiz, associated with CTX-M-15, in Freiburg and in Seoul, in Barcelona, associated with DHA-1.[21,39–41] ST17 belongs to the ST17 complex, which contains four single-locus variants and six double-locus variants.[41] *K. pneumoniae* ST834 strains were previously involved in bla_{KPC} dissemination in New Jersey.[42] Besides the low number of isolates, we have detected two news sequences types: ST1617 and ST1618.

In this study, all isolates producing bla_{CMY-4} and bla_{DHA-1} co-expressed the broad-spectrum TEM-1 β-lactamase and three of them co-produced CTX-M and/or SHV ESBL. This enzyme combination complicates their detection and treatment. bla_{CMY-4} gene was located on broad host range A/C conjugative plasmid which was among the most commonly reported worldwide. In the last decades, Inc A/C plasmids have been associated with the spread of the AmpC beta lactamase CMY-2, in strains isolated from human, beef, chicken, turkey, and pork, revealing that this common plasmid backbone is broadly disseminated among resistant zoonotic pathogens.[9,43]

In our study, the genetic organization of bla_{CMY-4} and its variants was highly conserved. All the isolates carried the transposon-like element IS*Ecp1* (IS*Ecp1*/ΔIS*Ecp1*-bla_{CMY}-*blc-sugE*), as documented previously.[44]

The bla_{DHA-1}gene was previously found on different plasmids of Inc groups A/C, FIA, FII, L/M, N, R and HI2 or of unknown Inc groups.[9,11,26,41,45] Nevertheless, it is worth noting that bla_{DHA-1} gene was located on LVPK conjugative plasmid. Linkage of bla_{DHA-1} and *qnr*B4 genes of similar structures has been described in isolates of *K. pneumoniae*.[8] The association among bla_{DHA-1}, *qnrB4*, and *aac(6′)-Ib-cr* was reported before.[46] The *K. pneumoniae* 413 strain in our study harbored a combination of β-lactamase genes (bla_{CTX-3}, bla_{DHA-1}, bla_{SHV-12} and bla_{TEM-1}), PMQR determinants (*qnrB4* gene) and aminoglycoside acetyltransferase gene (*aac(6′)-Ib*). Despite several investigations, we could not determine the origin of this multiresistant strain. To our knowledge, this is the first description of this association of genes including $bla_{CTX-M-3}$ in the same strain. Identification of the sequences surrounding the bla_{DHA-1} gene found an *ampR* gene included in a complex sul-1-type integron that was likely similar to those previously reported.[8]

Use of antibiotics in both humans and animals, the global mobility of populations, and food products perpetuate the spread of multiresistant bacterial clones and resistance genes. Early identification of these organisms is necessary as the appropriate treatment might reduce the spread of these resistant strains and consequently mortality of hospitalized patients can be reduced. This emphasizes the need for such enzymes detection for preventing this emerging resistance into hospitals and for controlling its spread within the community. That will avoid therapeutic failures and nosocomial outbreaks.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

We are grateful to J. Madoux for her contribution to this work. We thank the team of curators of the Institut Pasteur MLST databases for curating the data and making them publicly available at http://www.pasteur.fr/mlst.

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