Intercontinental spread of OXA-48 beta-lactamase-producing *Enterobacteriaceae* over a 11-year period, 2001 to 2011

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OXA-48 beta-lactamase producers are emerging as an important threat mostly in the Mediterranean area. We report here the molecular epidemiology of a collection of OXA-48 beta-lactamase-positive enterobacterial isolates (n=107) recovered from European and north-African countries between January 2001 and December 2011. This collection included 67 *Klebsiella pneumoniae*, 24 *Escherichia coli* and 10 *Enterobacter cloacae*. Using the EUCAST breakpoints, ninety-eight isolates (91.6%) were of intermediate susceptibility or resistant to ertapenem, whereas 66% remained susceptible to imipenem. Seventy-five per cent of the isolates co-produced an extended-spectrum beta-lactamase, most frequently CTX-M-15 (77.5%). Susceptibility testing to non-beta-lactam antibiotics showed that colistin, tigecycline, amikacin, and fosfomycin remain active against most of the isolates. Multilocus sequence typing indicated that the most common sequence types (ST) were ST101 and ST38 for *K. pneumoniae* and *E. coli*, respectively. The bla_{OXA-48} gene was located on a 62 kb IncL/M plasmid in 92.5% of the isolates, indicating that a single plasmid was mainly responsible for the spread of that gene. In addition, this study identified multiple cases of importation of OXA-48 beta-lactamase producers at least in Europe, and spread of OXA-48 beta-lactamase producers giving rise to an endemic situation, at least in France.

Introduction

Currently, an emergence of carbapenem resistance in *Enterobacteriaceae* is reported, mostly related to the spread of carbapenemases [1]. Those carbapenem-hydrolysing beta-lactamases belong to the Ambler class A (e.g. KPC), class B (e.g. IMP, VIM and NDM) [1], and class D (e.g. OXA-48 and its variants possessing weaker but significant carbapenemase activity) [2]. OXA-48 had first been identified from a clinical *Klebsiella pneumoniae* isolate recovered in Istanbul, Turkey, in 2001 [3]. The corresponding gene, namely bla_{OXA-48}, was then also identified in *Escherichia coli* and *Citrobacter freundii*, still in Turkey [4]. For several years, OXA-48 was identified only in Turkey, and almost all OXA-48 beta-lactamase producers were reported from patients hospitalised in Turkey or with a link to that country [4,5]. Since 2008, this gene has been identified in many other countries, most often in *K. pneumoniae* isolates [2,5-10]. OXA-48 is now identified in the Middle East and in North African countries, and those countries are considered as reservoirs of OXA-48 beta-lactamase producers [2]. In addition to sporadic cases, an increasing number of outbreaks due to OXA-48-producing *K. pneumoniae* are currently observed, not only in Turkey but also in Belgium, France, Greece, the Netherlands and Spain [2,11-13]. *K. pneumoniae* strains belonging to specific sequence types (ST), such as ST395 and ST101, have been involved in those outbreaks [12,14].

In order to gain further understanding of that phenomenon, our study aimed at comparing the genetic features of OXA-48 beta-lactamase-producing strains recovered from various countries by analysing an existing collection of 107 bla_{OXA-48}-positive enterobacterial isolates. The genetic context and the location of the bla_{OXA-48} gene were investigated, as well as resistance to broad-spectrum beta-lactams and non-beta-lactam antibiotics.

Methods

Bacterial isolates

A total of 107 OXA-48 beta-lactamase-producing enterobacterial isolates were investigated retrospectively. Enterobacteriacae producing OXA-48-like beta-lactamases were not included in this study. All isolates had been recovered from clinical specimens except a single isolate (one *Serratia marcescens* strain from an environmental water sample in Morocco), and had been received between January 2001 and December 2011 in our National Reference Laboratory which is also used as an International Reference Laboratory by many colleagues worldwide who send us their isolates for

further characterisation. Of identical strains in an outbreak, only one was included in this work. The distribution of clinical samples was as follows: rectal swabs (n=33), urine samples (n=24), blood samples (n=12), wound samples (n=7), respiratory specimens (n=4), catheters (n=4), bone specimens (n=2), peritoneal fluids (n=2), and placenta specimen (n=1). One sample per patient was included. Detailed information could not be obtained for 18 clinical samples. The isolates were identified to species level using the API 20E system (bioMérieux, La Balme-les-Grottes, France).

Susceptibility testing

Routine antibiograms were determined by disk diffusion method on Mueller-Hinton (MH) agar (Bio-Rad, Marnes-la-Coquette, France) and interpreted using the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (updated 2012) and of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for tigecycline and colistin [15,16]. In addition, MICs were determined for imipenem, meropenem, ertapenem, cefotaxime, and ceftazidime using E-test (bioMérieux, La Balme-les-Grottes, France). The production of extended-spectrum beta-lactamases (ESBL) was evidenced by a double-disk synergy test performed with cefepime, ceftazidime, and ticarcillin/clavulanic acid disks [17] and more recently by using the rapid ESBL NDP test [18].

PCR and sequencing of beta-lactamase-encoding genes

Whole-cell DNA was extracted using the QiaAmp DNA minikit and following the manufacturer's recommendations (Qiagen, Courtaboeuf, France). All isolates were screened by PCR for the Ambler class A and B carbapenemase-encoding genes bla_{KPC}, bla_{IMP}, bla_{VIM}, bla_{NDM} [19-20]. For each isolate, the bla_{OXA-48} gene was amplified using primers preOXA-48A and preOXA-48B, and subsequently sequenced [21]. Detection of other beta-lactamase genes such as bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{AmpC}-like, and bla_{OXA-1} was performed with internal primers, as described previously [19,22]. PCR products were analysed on agarose gel and sequenced by using the amplification primers with an automated sequencer (ABI PRISM 3100; Applied Biosystems). The nucleotide and deduced protein sequences were analysed using software from the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov).

Strain typing

Multilocus sequence typing (MLST) with seven housekeeping genes (*rpoB, gapA, mdh, pgi, phoE, infB* and *tonB*) was performed for *K. pneumoniae* isolates according to Diancourt et al. [23]. Allele sequences and STs were verified at http://pubmlst.org/Kpneumoniae. Fragments of seven housekeeping genes (*adk, fumC, gyrB, icd, mdh, purA* and *recA*) were amplified and sequenced for *E. coli* isolates as described on the following website http://mlst.ucc.ie/mlst/dbs/Ecoli. A different allele number was given to each distinct sequence within a locus, and a distinct ST number was attributed to each distinct combination of alleles. *E. coli* isolates were assigned to the major *E. coli* phylogenetic groups (A, B1, B2, and D) by multiplex PCR, as described [24]. The genetic relationship between the *Enterobacter cloacae* isolates was studied using Diversilab, a semi-automated typing system based on repetitive sequence-based PCR (rep-PCR) following the manufacturer's instructions (bioMérieux).

Plasmid DNA analysis, transformation and mating-out assays

Plasmid DNA was extracted from the isolates using the Kieser technique [25]. *E. coli* NCTC50192, harbouring four plasmids of 154, 66, 48 and 7 kb, was used as plasmid size marker. Plasmid DNAs were analysed by agarose gel electrophoresis. Direct transfer of the carbapenem resistance markers was attempted by liquid mating-out assays at 37°C, using *E. coli* J53 as recipient, or by electrotransformation of plasmid DNA, using *E. coli* TOP10 as recipient as reported [3,4]. Selection was performed on agar plates supplemented with ertapenem (0.5 µg/ml) and azide (100 µg/ml) for mating-out assays. In order to search for a possible chromosomal location of the bla_{OXA-48} gene in *E. coli* isolates 19 to 24, restriction with endonuclease I-*CeuI* followed by pulsed-field gel electrophoresis (PFGE) analysis was performed as described [26].

Replicon and transposon typing

PCR-based replicon typing (PBRT) of the main plasmid incompatibility groups reported in *Enterobacteriaceae* was performed as described [27] and using the specific primers designed from plasmid pOXA-48a [28]. Genetic structures surrounding the bla_{OXA-48} gene were determined according to the Tn*1999*-like PCR-mapping scheme as described [29].

Results

Bacterial isolates

A total of 107 isolates were studied, including *K. pneumoniae* (n=67), *E. coli* (n=24), and *E. cloacae* (n=10) (Table 1). Other enterobacterial species were identified: *Citrobacter koseri* (n=2), *C. freundii* (n=1), *Klebsiella oxytoca* (n=1), *Providencia rettgeri* (n=1), and *S. marcescens* (n=1). They had been isolated in France (n=61), Morocco (n=22), Turkey (n=11), Egypt (n=3), Lebanon (n=2), Tunisia (n=2), Switzerland (n=2), South Africa (n=2), Belgium (n=1), and the Netherlands (n=1), respectively (Table 1). Among the 61 strains collected in France, 30 had a history of international travel to the following countries: Morocco (n=14), Tunisia (n=2), Libya (n=5), Algeria (n=4), Egypt (n=3), Senegal (n=1), and Kuwait (n=1). In 12 cases, no travel history from a foreign country was identified. For the remaining 19 cases, no precise travel information could be obtained (Table 1).

Table 1A

Genetic features associated with OXA-48 beta-lactamase producers, 2001–11 (n=107)

Species		Country of isolation	Travel history	beta-lactams MICs (µg/mL)					Sequence type	Genetic location of bla_{OXA-48}	Incompatibility group of bla_{OXA-48}-positive plasmid	Non-beta-lactam-associated resistances	Associated beta-lactam resistance determinants[a]	Phylogenetic group	Transposon bearing bla_{OXA-48}
				ERT	IMP	MER	CAZ	CTX							
Enterobacter cloacae	1	France	Morocco	8	1	1	64	>256	ND	Plasmidic	IncL/M	Q, Gm, Tm, TET, Cm, SXT, FT	CTX-M-15, TEM-1, OXA-1	ND	Tn*1999.2*
E. cloacae	2	France	?	>32	1	1.5	32	>256	ND	Plasmidic	IncL/M	Q, Tm, Ak, TET, TGC, Cm, SXT, FT	CTX-M-15, TEM-1, OXA-1	ND	Tn*1999.2*
E. cloacae	3	France	None	4	0.5	0.5	>256	>256	ND	Plasmidic	IncL/M	Q, Ami, TET, TGC, Cm, Fos, SXT, FT	CTX-M-15, TEM-1, OXA-1 DHA-7, SHV-12	ND	Tn*1999.2*
E. cloacae	4	France	Algeria	3	0.75	0.38	48	>256	ND	Plasmidic	IncL/M	FOS	CTX-M-15	ND	Tn*1999.4*
E. cloacae	5	France	Morocco	16	1	0.75	32	>256	ND	Plasmidic	IncL/M	Q, Gm, Tm, Cm, SXT, FT	CTX-M-15, TEM-1, OXA-1	ND	Tn*1999.2*
E. cloacae	6	France	Morocco	1.5	0.5	0.25	16	>256	ND	Plasmidic	IncL/M	Q, Gm, Tm, TET, Cm, SXT	CTX-M-15, TEM-1, OXA-1	ND	Tn*1999.1*
E. cloacae	7	Morocco	None	1	0.5	0.38	0.5	8	ND	Plasmidic	IncL/M	Ami, TET, SXT, FT	CTX-M-9, TEM-1	ND	Tn*1999.2*
E. cloacae	8	Morocco	None	1	0.5	0.38	48	16	ND	Plasmidic	IncL/M	OFX, Ami, TET, SXT, FT	CTX-M-9, TEM-1, SHV-12	ND	Tn*1999.2*
E. cloacae	9	Morocco	None	0.75	0.38	0.19	8	128	ND	Plasmidic	IncL/M	Gm, Tm, TET, Cm, SXT, FT	CTX-M-15, TEM-1	ND	Tn*1999.2*
E. cloacae	10	Morocco	None	6	1	1	48	16	ND	Plasmidic	IncL/M	OFX, Gm, Tm, Cm, SXT, FT	TEM-1, DHA-7, SHV-12	ND	Tn*1999.2*
C. koseri	11	France	?	2	0.38	0.38	0.38	2	ND	Plasmidic	IncL/M	None	None	ND	Tn*1999.2*
C. koseri	12	France	?	2	0.75	0.38	2	2	ND	Plasmidic	IncL/M	None	None	ND	Tn*1999.2*
Klebsiella oxytoca	13	Morocco	None	4	1	0.5	256	256	ND	Plasmidic	IncL/M	Q, Gm, Tm, TET, Cm, SXT	CTX-M-15, TEM-1	ND	Tn*1999.2*
Citrobacter freundii	14	France	None	4	0.75	0.38	64	12	ND	Plasmidic	IncL/M	Q, Gm, Tm, TET, TGC, Cm, SXT	SHV-12, TEM-1	ND	Tn*1999.2*
Providencia rettgeri	15	Turkey	None	>32	>32	>32	32	16	ND	Plasmidic	Inc A/C	Q, Gm, Tm, TET, Cm, SXT, FT	TEM-101	ND	Tn*1999.1*
Serratia marcescens	16	Morocco	None	>32	8	4	1	3	ND	Plasmidic	IncL/M	Q, TET, Cm, SXT, FT	OXA-1	ND	Tn*1999.1*
E. coli	17	France	Algeria	0.5	0.38	0.12	16	>256	10	Plasmidic	IncL/M	Q, SXT	CTX-M-15, TEM-1	A	Tn*1999.4*

Ak: amikacin; Ami: aminoglycosides; Cm: chloramphenicol; Cs: colistin; △Tn*1999*: truncated transposon Tn*1999*; FOS: fosfomycin; FT: nitrofurantoin; Gm gentamicin; MIC: minimum inhibitory concentration; ND: not determinable; OFX: ofloxacin; Q: fluoroquinolones; SXT: sulfamethoxazole-trimethoprim; TET: tetracycline; TGC: tigecycline; Tm: tobramycin.

[a] Resistance markers being co-harboured by the bla_{OXA-48}-carrying plasmid are underlined.

Table 1B

Genetic features associated with OXA-48 beta-lactamase producers, 2001–11 (n=107)

Species		Country of isolation	Travel history	beta-lactams MICs (µg/mL)					Sequence type	Genetic location of bla_{OXA-48}	Incompatibility group of bla_{OXA-48}-positive plasmid	Non-beta-lactam-associated resistances	Associated beta-lactam resistance determinants[a]	Phylogenetic group	Transposon bearing bla_{OXA-48}
				ERT	IMP	MER	CAZ	CTX							
E. coli	18	France	Libya	1	0.5	0.19	›256	32	10	Plasmidic	IncL/M	Ak, Tm, TET, Cm, SXT	CMY-2, VEB-8, TEM-1	A	Tn*1999.2*
E. coli	19	France	Egypt	3	0.5	0.19	0.5	24	38	Chromosomic	ND	OFX, Gm, Tm, SXT	CTX-M-24, TEM-1	D	△Tn*1999.2*
E. coli	20	France	Turkey	3	0.75	0.38	0.75	24	38	Chromosomic	ND	OFX, Gm, Tm, SXT	CTX-M-24, TEM-1	D	△Tn*1999.2*
E. coli	21	France	Egypt	0.5	0.25	0.19	0.5	24	38	Chromosomic	ND	OFX, Gm, Tm, SXT	CTX-M-24, TEM-1	D	△Tn*1999.2*
E. coli	22	France	None	1	0.38	0.25	0.5	24	38	Chromosomic	ND	OFX, Gm, Tm, SXT	CTX-M-24, TEM-1	D	△Tn*1999.2*
E. coli	23	Switzerland	?	8	0.75	1	1.5	48	38	Chromosomic	ND	OFX, Gm, Tm, SXT	CTX-M-24, TEM-1	D	△Tn*1999.2*
E. coli	24	Egypt	None	2	0.5	0.25	0.5	24	38	Chromosomic	ND	OFX, Gm, Tm, SXT	CTX-M-24, TEM-1	D	△Tn*1999.2*
E. coli	25	Lebanon	None	2	0.75	0.25	1.5	48	38	Plasmidic	IncL/M	SXT	CTX-M-14, TEM-1	D	Tn*1999.2*
E. coli	26	France	?	0.5	0.38	0.12	0.12	0.09	46	Plasmidic	IncL/M	Q, TET, SXT	TEM-1	A	Tn*1999.2*
E. coli	27	France	Egypt	0.5	0.5	0.19	8	›256	69	Plasmidic	IncL/M	TET, SXT	CTX-M-15, TEM-1	D	Tn*1999.2*
E. coli	28	France	None	0.75	0.5	0.25	0.12	0.38	95	Plasmidic	IncL/M	None	TEM-1	B2	Tn*1999.2*
E. coli	29	Egypt	None	›32	32	›32	›256	256	101	Plasmidic	IncL/M	Q, Ami, TET, Cm, SXT	TEM-1, VIM-1, CMY-4	B1	Tn*1999.1*
E. coli	30	France	?	3	1.5	0.5	0.19	1	362	Plasmidic	IncL/M	TET, SXT	TEM-1	D	Tn*1999.2*
E. coli	31	France	Morocco	2	0.38	0.25	0.5	0.5	410	Plasmidic	IncL/M	Q, TET	None	A	Tn*1999.2*
E. coli	32	France	?	0.75	0.38	0.19	16	128	617	Plasmidic	IncL/M	Q, Gm, Tm, TET, SXT	CTX-M-15, OXA-1	A	Tn*1999.2*
E. coli	33	France	?	0.75	0.38	0.19	8	64	617	Plasmidic	IncL/M	Q, Gm, Tm, TET, SXT	CTX-M-15, OXA-1	A	Tn*1999.2*
E. coli	34	Turkey	None	24	1.5	12	16	192	648	Plasmidic	IncL/M	Q, Gm, Tm, TET, SXT	CTX-M-15, TEM-1, OXA-1	D	Tn*1999.1*
E. coli	35	Turkey	None	›32	1.5	12	16	256	648	Plasmidic	IncL/M	Q, Gm, Tm, TET, SXT	CTX-M-15, TEM-1, OXA-1	D	Tn*1999.1*
E. coli	36	France	Morocco	1.5	0.38	0.25	24	›256	746	Plasmidic	IncL/M	Q, Gm, Tm, TET, Cm, SXT	CTX-M-15, TEM-1, OXA-1	A	Tn*1999.1*
E. coli	37	France	?	1.5	0.5	0.19	24	256	963	Plasmidic	Inc F	None	CTX-M-15, TEM-1	D	△Tn*1999.1*
E. coli	38	France	?	1	0.5	0.19	0.12	0.75	1092	Plasmidic	IncL/M	None	None	B2	Tn*1999.2*
E. coli	39	France	None	1	0.5	0.19	1.5	24	2969	Plasmidic	IncL/M	TET	CTX-M-15	D	Tn*1999.2*

Ak: amikacin; Ami: aminoglycosides; Cm: chloramphenicol; Cs: colistin; △Tn*1999*: truncated transposon Tn*1999*; FOS: fosfomycin; FT: nitrofurantoin; Gm gentamicin; MIC: minimum inhibitory concentration; ND: not determinable; OFX: ofloxacin; Q: fluoroquinolones; SXT: sulfamethoxazole-trimethoprim; TET: tetracycline; TGC: tigecycline; Tm: tobramycin.

[a] Resistance markers being co-harboured by the bla_{OXA-48}-carrying plasmid are underlined.

Table 1c

Genetic features associated with OXA-48 beta-lactamase producers, 2001–11 (n=107)

Species		Country of isolation	Travel history	beta-lactams MICs (µg/mL)					Sequence type	Genetic location of bla_{OXA-48}	Incompatibility group of bla_{OXA-48}-positive plasmid	Non-beta-lactam-associated resistances	Associated beta-lactam resistance determinants[a]	Phylogenetic group	Transposon bearing bla_{OXA-48}
				ERT	IMP	MER	CAZ	CTX							
E. coli	40	France	Morocco	0.75	0.5	0.19	1	16	2969	Plasmidic	IncL/M	TET	CTX-M-15	D	Tn*1999.2*
K. pneumoniae	41	Morocco	None	6	0.75	0.75	192	256	11	Plasmidic	IncL/M	Q, Ami, TET, Cm, SXT, FT	CTX-M-15, TEM-1	ND	Tn*1999.2*
K. pneumoniae	42	Turkey	None	›32	›32	›32	›256	64	14	Plasmidic	IncL/M	Q, Ami, Cm, SXT	SHV-2a , TEM-1, OXA-47	ND	Tn*1999.1*
K. pneumoniae	43	Turkey	None	›32	2	4	256	48	14	Plasmidic	IncL/M	Q, Ak, Tm, FT	SHV-12, TEM-1, OXA-1	ND	Tn*1999.1*
K. pneumoniae	44	Turkey	None	›32	›32	›32	›256	48	14	Plasmidic	IncL/M	Q, Ami, Cm, FOS	OXA-1, TEM-1, SHV-12	ND	Tn*1999.1*
K. pneumoniae	45	Egypt	None	1.5	2	0.75	0.19	1.5	14	Plasmidic	IncL/M	FT	OXA-1	ND	Tn*1999.2*
K. pneumoniae	46	France	?	›32	8	6	1	3	15	Plasmidic	IncL/M	Q, Tm, TET, TGC, Cm, FOS, SXT, FT	TEM-1	ND	Tn*1999.2*
K. pneumoniae	47	France	Morocco	2	0.5	0.5	192	›256	15	Plasmidic	IncL/M	Q, Gm, Tm, SXT, FT	CTX-M-15, TEM-1, OXA-1	ND	Tn*1999.2*
K. pneumoniae	48	France	Morocco	2	1	0.5	48	128	15	Plasmidic	IncL/M	Q, Gm, Tm, SXT, FT	CTX-M-15, TEM-1, OXA-1	ND	Tn*1999.2*
K. pneumoniae	49	Morocco	None	12	1	2	8	6	15	Plasmidic	IncL/M	Q, Ami, TET, TGC, Cm, FOS, SXT, FT	DHA-1, TEM-1	ND	Tn*1999.2*
K. pneumoniae	50	Morocoo	None	›32	›32	›32	256	›256	15	Plasmidic	IncL/M	Q, Gm, Tm, TET, FOS, SXT, FT	CTX-M-15, TEM-1	ND	Tn*1999.1*
K. pneumoniae	51	Morocco	None	12	1	2	8	6	15	Plasmidic	IncL/M	Q, Ami, TET, TGC, Cm, FOS, SXT, FT	DHA-1, TEM-1	ND	Tn*1999.2*
K. pneumoniae	52	Morocco	None	2	0.38	0.5	24	›256	15	Plasmidic	IncL/M	Q, Gm, Tm, SXT, FT	CTX-M-15, TEM-1	ND	Tn*1999.2*
K. pneumoniae	53	Turkey	None	2	0.5	0.5	64	›256	16	Plasmidic	IncL/M	Q, Tm, Ak, TET, SXT, FT	CTX-M-15, TEM-1, OXA-1	ND	Tn*1999.2*
K. pneumoniae	54	Morocco	None	0.38	0.38	0.5	12	96	25	Plasmidic	IncL/M	Q, Gm, Tm, TET, TGC, SXT, FT	CTX-M-15, TEM-1, OXA-1	ND	Tn*1999.2*
K. pneumoniae	55	Morocco	None	0.38	0.38	0.5	12	96	25	Plasmidic	IncL/M	Q, Gm, Tm, TET, SXT, FT	CTX-M-15, TEM-1	ND	Tn*1999.1*
K. pneumoniae	56	France	Koweit	›32	32	24	1.5	2	29	Plasmidic	IncL/M	OFX, TET, TGC, Cm, FOS, SXT, FT	None	ND	Tn*1999.2*

Ak: amikacin; Ami: aminoglycosides; Cm: chloramphenicol; Cs: colistin; △Tn*1999*: truncated transposon Tn*1999*; FOS: fosfomycin; FT: nitrofurantoin; Gm gentamicin; MIC: minimum inhibitory concentration; ND: not determinable; OFX: ofloxacin; Q: fluoroquinolones; SXT: sulfamethoxazole-trimethoprim; TET: tetracycline; TGC: tigecycline; Tm: tobramycin.

[a] Resistance markers being co-harboured by the bla_{OXA-48}-carrying plasmid are underlined.

Table 1D

Genetic features associated with OXA-48 beta-lactamase producers, 2001–11 (n=107)

Species		Country of isolation	Travel history	beta-lactams MICs (µg/mL)					Sequence type	Genetic location of bla_{OXA-48}	Incompatibility group of bla_{OXA-48}-positive plasmid	Non-beta-lactam-associated resistances	Associated beta-lactam resistance determinants[a]	Phylogenetic group	Transposon bearing bla_{OXA-48}
				ERT	IMP	MER	CAZ	CTX							
K. pneumoniae	57	France	Morocco	0.5	0.38	0.25	0.19	0.38	35	Plasmidic	IncL/M	None	None	ND	Tn*1999.2*
K. pneumoniae	58	France	None	0.75	0.5	0.25	0.09	0.38	37	Plasmidic	IncL/M	TET, FT	None	ND	Tn*1999.2*
K. pneumoniae	59	France	?	1	0.5	0.25	0.12	0.5	45	Plasmidic	IncL/M	None	TEM-1	ND	Tn*1999.2*
K. pneumoniae	60	France	?	1	0.5	0.25	0.12	0.25	45	Plasmidic	IncL/M	FOS	TEM-1	ND	Tn*1999.2*
K. pneumoniae	61	France	?	0.75	0.5	0.25	0.19	0.25	45	Plasmidic	IncL/M	None	TEM-1	ND	Tn*1999.2*
K. pneumoniae	62	France	None	4	4	1	0.5	0.5	45	Plasmidic	IncL/M	TET, FOS	None	ND	Tn*1999.2*
K. pneumoniae	63	Tunisia	None	>32	1.5	12	48	>256	101	Plasmidic	IncL/M	Q, Gm, Tm, TET, Cm, SXT, FT	CTX-M-15, TEM-1, OXA-1	ND	Tn*1999.2*
K. pneumoniae	64	France	?	>32	24	16	32	>256	101	Plasmidic	IncL/M	Q, Gm, Tm, TET, FOS, SXT, FT	CTX-M-15, TEM-1, OXA-1	ND	Tn*1999.2*
K. pneumoniae	67	Tunisia	None	>32	2	8	>256	>256	101	Plasmidic	IncL/M	Ami, TET, Cm, FOS, SXT, FT	CTX-M-15, TEM-1	ND	Tn*1999.2*
K. pneumoniae	68	France	None	3	0.5	0.38	48	96	101	Plasmidic	IncL/M	Q, Gm, Tm, TET, SXT, FT	CTX-M-15, TEM-1, OXA-1	ND	Tn*1999.2*
K. pneumoniae	69	Switzerland	?	6	0.5	0.75	48	>256	101	Plasmidic	IncL/M	Q, Tm, Ak, TET, SXT, FT, TGC	CTX-M-15, OXA-1	ND	Tn*1999.2*
K. pneumoniae	70	France	Morocco	>32	3	16	192	>256	101	Plasmidic	IncL/M	Q, Gm, Tm, TET, FOS, SXT, FT	CTX-M-15, TEM-1, OXA-1	ND	Tn*1999.2*
K. pneumoniae	71	France	None	4	0.5	0.5	48	128	101	Plasmidic	IncL/M	Q, Tm, SXT, FT	CTX-M-15, OXA-1	ND	Tn*1999.2*
K. pneumoniae	72	France	Libya	>32	3	8	>256	>256	101	Plasmidic	IncL/M	Q, Gm, Tm, TET, SXT, FT	CTX-M-15, TEM-1, OXA-1	ND	Tn*1999.2*
K. pneumoniae	73	France	Libya	>32	>32	>32	>256	>256	101	Plasmidic	IncL/M	Q, Gm, Tm, TET, Cm, FOS, SXT, FT	CTX-M-15, TEM-1, OXA-1	ND	Tn*1999.2*
K. pneumoniae	74	South Africa	?	>32	3	8	>256	>256	101	Plasmidic	IncL/M	Q, Gm, Tm, TET, TGC, Cm, SXT, FT	CTX-M-15, OXA-1	ND	Tn*1999.2*
K. pneumoniae	75	South Africa	?	>32	3	8	>256	>256	101	Plasmidic	IncL/M	Q, Gm, Tm, TET, Cs, SXT, FT	CTX-M-15, OXA-1	ND	Tn*1999.2*
K. pneumoniae	76	Morocco	None	>32	>32	16	192	>256	101	Plasmidic	IncL/M	Q, Gm, Tm, TET, FOS, SXT, FT	CTX-M-15, TEM-1	ND	Tn*1999.2*
K. pneumoniae	77	Morocco	None	>32	>32	>32	256	>256	101	Plasmidic	IncL/M	Q, Tm, Ak, TET, FOS, SXT, FT	CTX-M-15, TEM-1	ND	Tn*1999.2*

Ak: amikacin; Ami: aminoglycosides; Cm: chloramphenicol; Cs: colistin; △Tn*1999*: truncated transposon Tn*1999*; FOS: fosfomycin; FT: nitrofurantoin; Gm gentamicin; MIC: minimum inhibitory concentration; ND: not determinable; OFX: ofloxacin; Q: fluoroquinolones; SXT: sulfamethoxazole-trimethoprim; TET: tetracycline; TGC: tigecycline; Tm: tobramycin.

[a] Resistance markers being co-harboured by the bla_{OXA-48}-carrying plasmid are underlined.

Table 1E

Genetic features associated with OXA-48 beta-lactamase producers, 2001–11 (n=107)

Species		Country of isolation	Travel history	beta-lactams MICs (µg/mL)					Sequence type	Genetic location of bla_{OXA-48}	Incompatibility group of bla_{OXA-48}-positive plasmid	Non-beta-lactam-associated resistances	Associated beta-lactam resistance determinants[a]	Phylogenetic group	Transposon bearing bla_{OXA-48}
				ERT	IMP	MER	CAZ	CTX							
K. pneumoniae	78	Morocco	None	2	0.38	0.5	192	256	101	Plasmidic	IncL/M	Q, Gm, Tm, TET, SXT, FT	CTX-M-15, TEM-1, OXA-1	ND	Tn*1999.2*
K. pneumoniae	79	Morocco	None	2	0.38	0.5	>256	>256	101	Plasmidic	IncL/M	Q, Tm, Ak, TET, SXT, FT	CTX-M-15, TEM-1, OXA-1	ND	Tn*1999.2*
K. pneumoniae	80	Belgium	None	4	1	1	1	0.5	147	Plasmidic	IncL/M	Q, TET, TGC, Cm, SXT	None	ND	Tn*1999.2*
K. pneumoniae	81	Turkey	None	>32	32	8	96	>256	147	Plasmidic	IncL/M	Q, Tm, Ak, TET, TGC, Cm, FOS, SXT, FT	CTX-M-15, TEM-1, OXA-1	ND	Tn*1999.2*
K. pneumoniae	82	France	Tunisia	3	0.38	0.38	64	>256	147	Plasmidic	IncL/M	Q, Gm, Tm, TET, TGC, Cm, SXT, FT	CTX-M-15, TEM-1, OXA-1	ND	Tn*1999.2*
K. pneumoniae	83	France	?	2	0.38	0.38	96	192	147	Plasmidic	IncL/M	Q, Tm, Ak, FT	CTX-M-15, TEM-1	ND	Tn*1999.2*
K. pneumoniae	84	France	Libya	3	1.5	3	>256	>256	147	Plasmidic	IncL/M	Q, Gm, Tm, Ak, TET, Cm, SXT, FT	CTX-M-15, TEM-1, OXA-1, CMY-2	ND	Tn*1999.2*
K. pneumoniae	85	France	Libya	>32	3	8	>256	>256	147	Plasmidic	IncL/M	Q, Ami, TET, Cm, FOS, SXT, FT	CTX-M-15, TEM-1, OXA-1, CMY-2	ND	Tn*1999.2*
K. pneumoniae	86	France	?	1	0.25	0.25	48	>256	307	Plasmidic	IncL/M	Q, Gm, Tm, TET, Cm, SXT, FT	CTX-M-15, TEM-1, OXA-1	ND	Tn*1999.2*
K. pneumoniae	87	France	Algeria	0.5	0.38	0.19	32	192	336	Plasmidic	IncL/M	Q, Tm, Ak, SXT	CTX-M-15, OXA-1	ND	Tn*1999.2*
K. pneumoniae	88	Morocoo	None	1	0.5	0.5	8	48	392	Plasmidic	IncL/M	Q, Gm, Tm, TET, SXT, FT	CTX-M-15, TEM-1	ND	Tn*1999.2*
K. pneumoniae	89	Morocco	None	12	6	3	24	>256	392	Plasmidic	IncL/M	Q, Gm, Tm TET, Cm, FOS, SXT, FT	CTX-M-15, TEM-1, OXA-1	ND	Tn*1999.2*
K. pneumoniae	90	France	None	3	0.75	0.75	96	>256	395	plasmidic	IncL/M	Q, Tm, Gm, TET, FOS, SXT, FT, Cm	CTX-M-15, TEM-1, OXA-1	ND	Tn*1999.2*
K. pneumoniae	91	France	None	2	0.5	0.38	1	1	395	Plasmidic	IncL/M	Q, Tm, Ak, TET, TGC, Cm, FOS, SXT, FT	OXA-1	ND	Tn*1999.2*
K. pneumoniae	92	Netherlands	None	2	0.5	0.38	32	192	395	Plasmidic	IncL/M	Q, Gm, Tm, TET, Cm, FOS, SXT, FT	CTX-M-15, TEM-1, OXA-1	ND	Tn*1999.2*
K. pneumoniae	93	France	Morocco	2	0.38	0.38	256	256	395	Plasmidic	IncL/M	Q, Gm, Tm, TET, Cm, FOS, SXT, FT	CTX-M-15, TEM-1, OXA-1	ND	Tn*1999.2*

Ak: amikacin; Ami: aminoglycosides; Cm: chloramphenicol; Cs: colistin; △Tn*1999*: truncated transposon Tn*1999*; FOS: fosfomycin; FT: nitrofurantoin; Gm gentamicin; MIC: minimum inhibitory concentration; ND: not determinable; OFX: ofloxacin; Q: fluoroquinolones; SXT: sulfamethoxazole-trimethoprim; TET: tetracycline; TGC: tigecycline; Tm: tobramycin.

[a] Resistance markers being co-harboured by the bla_{OXA-48}-carrying plasmid are underlined.

Table 1f

Genetic features associated with OXA-48 beta-lactamase producers, 2001–11 (n=107)

Species		Country of isolation	Travel history	beta-lactams MICs (µg/mL)					Sequence type	Genetic location of *bla*$_{OXA-48}$	Incompatibility group of *bla*$_{OXA-48}$-positive plasmid	Non-beta-lactam-associated resistances	Associated beta-lactam resistance determinants[a]	Phylogenetic group	Transposon bearing *bla*$_{OXA-48}$
				ERT	IMP	MER	CAZ	CTX							
K. pneumoniae	94	France	Morocco	›32	24	32	128	›256	395	Plasmidic	IncL/M	Q, Gm, Tm, TET, TGC, Cm, FOS, SXT, FT	CTX-M-15, TEM-1, OXA-1	ND	Tn*1999.2*
K. pneumoniae	95	Morocco	None	3	0.5	0.5	›256	›256	395	Plasmidic	IncL/M	Q, Gm, Tm, TET, TGC, Cm, FOS, SXT, FT	CTX-M-15, TEM-1	ND	Tn*1999.2*
K. pneumoniae	96	Morocoo	None	3	0.5	0.5	›256	›256	395	Plasmidic	IncL/M	Q, Gm, Tm, TET, Cm, FOS, SXT, FT	CTX-M-15, TEM-1, OXA-1	ND	Tn*1999.2*
K. pneumoniae	97	France	Morocco	1.5	3	0.25	32	96	405	Plasmidic	IncL/M	Q, Gm, Tm, TET, FOS, SXT, FT	CTX-M-15, TEM-1, OXA-1	ND	Tn*1999.1*
K. pneumoniae	98	Lebanon	None	›32	›32	›32	0.38	3	496	Plasmidic	IncL/M	FOS, SXT, FT	None	ND	Tn*1999.2*
K. pneumoniae	99	France	Algeria	1	0.38	0.19	0.25	0.25	530	Plasmidic	IncL/M	FT	TEM-1	ND	Tn*1999.2*
K. pneumoniae	100	France	?	4	0.75	0.5	0.5	0.75	685	Plasmidic	IncL/M	FT	None	ND	Tn*1999.2*
K. pneumoniae	101	Turkey	?	0.38	0.38	0.12	0.09	0.12	981	Plasmidic	IncL/M	OFX	None	ND	Tn*1999.2*
K. pneumoniae	102	Turkey	None	›32	›32	32	32	›256	982	Plasmidic	IncL/M	OFX, Tm, TET, TGC, FOS, SXT, FT	CTX-M-15, OXA-1	ND	Tn*1999.2*
K. pneumoniae	103	Turkey	None	›32	2	6	›256	48	984	Plasmidic	IncL/M	Q, Gm, Tm, TET, Cm, SXT, FT	SHV-12, TEM-1	ND	Tn*1999.1*
K. pneumoniae	104	France	Senegal	1	0.5	0.25	12	48	985	Plasmidic	IncL/M	OFX, Tm, Gm, TET, TGC, Cm, FOS, SXT	CTX-M-15, TEM-1, OXA-1	ND	Tn*1999.2*
K. pneumoniae	105	France	Morocco	3	0.5	0.38	96	256	986	Plasmidic	IncL/M	Q, Gm, Tm, TET, Cm, SXT, FT	CTX-M-15, TEM-1, OXA-1	ND	Tn*1999.2*
K. pneumoniae	106	France	?	3	0.5	0.38	1.5	0.75	987	Plasmidic	IncL/M	OFX, TET, TGC, Cm, FT	None	ND	Tn*1999.2*
K. pneumoniae	107	Morocco	None	1	0.38	0.5	12	3	1027	Plasmidic	IncL/M	Q, Ami, TET, Cm, FOS, SXT, FT	TEM-1, SHV-27, DHA-1	ND	Tn*1999.2*

Ak: amikacin; Ami: aminoglycosides; Cm: chloramphenicol; Cs: colistin; △Tn*1999*: truncated transposon Tn*1999*; FOS: fosfomycin; FT: nitrofurantoin; Gm gentamicin; MIC: minimum inhibitory concentration; ND: not determinable; OFX: ofloxacin; Q: fluoroquinolones; SXT: sulfamethoxazole-trimethoprim; TET: tetracycline; TGC: tigecycline; Tm: tobramycin.

[a] Resistance markers being co-harboured by the *bla*$_{OXA-48}$-carrying plasmid are underlined.

Table 2

Susceptibility to carbapenems of *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter cloacae* isolates, 2001–11 (n=107)

Species	Antimicrobial drug	Susceptibility Number (%) of isolates		
		Susceptible	Intermediate	Resistant
K. pneumoniae (n=67)	Imipenem	40 (60)	13 (19)	14 (21)
	Ertapenem	5 (7)	9 (13)	53 (79)
	Meropenem	40 (60)	6 (9)	21 (31)
E. coli (n=24)	Imipenem	20 (84)	3 (12)	1 (4)
	Ertapenem	4 (17)	8 (33)	12 (50)
	Meropenem	21 (88)	0 (0)	3 (12)
E. cloacae (n=10)	Imipenem	10 (100)	0 (0)	0 (0)
	Ertapenem	0 (0)	3 (30)	7 (70)
	Meropenem	9 (90)	1 (10)	0 (0)

The percentages are rounded so as to add up to 100%.

The results in this Table are from E-tests.

Susceptibility to carbapenems and broad-spectrum cephalosporins

Results of susceptibility testing are shown in Tables 2 and 3. According to the CLSI guidelines, 40 (60%) *K. pneumoniae* isolates, 20 (83%) *E. coli* isolates, and 10 (100%) *E. cloacae* isolates were susceptible to imipenem (Table 2). In addition, 40 (60%) *K. pneumoniae*, 21 (88%) *E. coli*, and 9 (90%) *E. cloacae* isolates were susceptible to meropenem. By contrast, 62 (92%) *K. pneumoniae*, 20 (83%) *E. coli*, and 10 (100%) *E. cloacae* isolates were found of intermediate susceptibility or resistant to ertapenem (Table 2). Regarding the broad-spectrum cephalosporins, 73 (68%) and 90 (84%) isolates were resistant or of intermediate susceptibility to ceftazidime and cefotaxime, respectively (Table 1 and 3).

Beta-lactamase genes

Among the 107 OXA-48-producing isolates, 80 (75%) co-produced an ESBL. A $bla_{\text{CTX-M}}$-like gene was detected in 71 (66%) of the isolates (89% of the ESBL-producing isolates). Among the different CTX-M variants identified, those belonging to the CTX-M-1 and CTX-M-9 groups accounted for 87.5% (n=62) and 12.5% (n=9), respectively. CTX-M-15 was the only representative of the CTX-M-1 group. In the CTX-M-9 group, the $bla_{\text{CTX-M-14}}$ was identified in a single *E. coli* (Table 1). Two *E. cloacae* isolates harboured a $bla_{\text{CTX-M-9}}$ gene and 6 *E. coli* isolates harboured a $bla_{\text{CTX-M-24}}$ gene. The other ESBL determinants were SHV-2a (one *K. pneumoniae*), SHV-12 (three *K. pneumoniae*, two *E. cloacae*, and one *C. freundii*), SHV-27 (one *K. pneumoniae*) and TEM-101 (*P. rettgeri* isolate no. 15). Among the SHV-12-producing isolates, one *E. cloacae* co-produced CTX-M-9 (Table 1). In addition, a novel VEB variant, namely VEB-8, was identified in a single *E. coli* isolate from Libya that co-produced CMY-2 (*E. coli* 18). VEB-8 differed from VEB-5 by a single amino acid substitution (GenBank accession number JX679208) [30,31]. It is interesting to note that ESBLs were not related to date or geographic area of isolation.

Furthermore, nine isolates (8.5%) co-produced a plasmid-mediated AmpC-type beta-lactamase. Four isolates (3.8%) produced a CMY-type beta-lactamase, namely CMY-4 in a single *E. coli* isolate from Egypt and CMY-2 in three isolates (a single *E. coli* and two *K. pneumoniae* isolates from Libya). Five isolates (5%) produced a DHA-like AmpC, namely DHA-1 in three *K. pneumoniae* isolates and DHA-7 in two *E. cloacae* isolates. All the DHA-producing isolates originated from Morocco. A single isolate (*E. coli* 29 from Egypt) co-produced OXA-48 and another carbapenemase, namely VIM-1, in addition to CMY-4. The non-ESBL beta-lactamases TEM-1 and OXA-1 were detected in 79 (74%) and 47 (44%) isolates, respectively.

Susceptibility to non-beta-lactam antibiotics

Results of susceptibility testing for non-beta-lactam antibiotics are shown in Table 3. Four antibiotics were active against the majority of the isolates; 104 (99%) of the 107 isolates were susceptible to colistin, 90 (84.1%) to tigecycline, 83 (77.6%) to amikacin, and 77 (72%) to fosfomycin. Conversely, 84 (78.5%) of the 107 isolates were resistant to sulfamethoxazole-trimethoprim, 72 (67.3%) to tetracycline, 64 (59.8%) to ciprofloxacin, and 61 (57%) to gentamicin. Resistant isolates that produced an ESBL were mostly resistant also to non-beta-lactam antibiotics (Table 3).

Table 3

Susceptibility of the study isolates determined by disk diffusion method, 2001–11 (n=107)

Antimicrobial drug	Susceptibility Number (%) of isolates								
	Susceptible			Intermediate			Resistant		
	Total	ESBL	Non-ESBL	Total	ESBL	Non-ESBL	Total	ESBL	Non-ESBL
Ceftazidime	34 (31.8)	10 (9.4)	24 (22.4)	9 (8.4)	7 (6.5)	2 (1.9)	64 (59.8)	63 (58.9)	1 (0.9)
Cefotaxime	17 (15.9)	0 (0)	17 (15.9)	8 (7.5)	1 (1.0)	7 (6.5)	82 (76.6)	79 (73.8)	3 (2.8)
Tetracycline	34 (31.8)	21 (19.6)	13 (12.2)	1 (0.9)	1 (0.9)	0 (0)	72 (67.3)	58 (54.2)	14 (13.1)
Tigecycline	90 (84.1)	69 (64.5)	21 (19.6)	5 (4.7)	4 (3.7)	1 (1.0)	12 (11.2)	7 (6.5)	5 (4.7)
Fosfomycin	77 (72.0)	58 (54.2)	19 (17.8)	2 (1.8)	2 (1.8)	0 (0)	28 (26.2)	20 (18.7)	8 (7.5)
Sulfamethoxazol/ trimethoprim	23 (21.5)	7 (6.5)	16 (15.0)	0 (0)	0 (0)	0 (0)	84 (78.5)	73 (68.2)	11 (10.3)
Colistin	104[a] (99.0)	78 (74.3)	26 (24.7)	0 (0)	0 (0)	0 (0)	1 (1.0)	1 (1.0)	0 (0)
Ciprofloxacin	39 (36.4)	21 (19.6)	18 (16.8)	4 (3.8)	4 (3.8)	0 (0)	64 (59.8)	55 (51.4)	9 (8.4)
Amikacin	83 (77.6)	60 (56.1)	23 (21.5)	15 (14.0)	14 (13.1)	1 (0.9)	9 (8.4)	6 (5.6)	3 (2.8)
Gentamicin	43 (40.2)	19 (17.8)	24 (22.4)	3 (2.8)	3 (2.8)	0 (0)	61 (57.0)	58 (54.2)	3 (2.8)

ESBL: extended-spectrum beta-lactamases.

[a] The *Providencia rettgeri* and the *Serratia marcescens* isolates were excluded because of their natural resistance to colistin.

The percentages are rounded so as to add up to 100%.

Phylogenetic groups of the Escherichia coli isolates

More than half of *E. coli* isolates belonged to the phylogenetic group D (14 of the 24 *E. coli* isolates), seven *E. coli* isolates belonged to the phylogenetic group A, two belonged to the phylogenetic group B2, and one isolate belonged to the phylogenetic group B1 (Table 1).

Mulilocus sequence typing

The distribution of the sequence types among the *K. pneumoniae* and *E. coli* isolates is shown in Figures 1 and 2, respectively. ST101 was the most commonly observed ST for the *K. pneumoniae* isolates, accounting for 17 out of 67 isolates (25.4%), followed by ST395 and ST15 (7 isolates, 10.5%) (Figure 1). Six isolates (9%) belonged to ST147 (9%) and the other isolates to diverse STs, namely ST14 (n=4), ST45 (n=4), ST25 (n=2), ST392 (n=2), and one to other STs (Figure 1). Among the 24 OXA-48-positive *E. coli* isolates, seven belonged to ST38 (29.2%). The remaining 17 isolates belonged to STs 10, 617, 648 and 2969 (two isolates each) and to STs 46, 69, 95, 101, 362, 410, 746, 963 and 1092 (one isolate each) (Figure 2).

Since no MLST system has been developed for typing the *E. cloacae* species, these isolates were genotyped using the DiversiLab method. *E. cloacae* 7 and 9 recovered from Morocco were closely related, and *E. cloacae* 1, 2 and 5 (also from Morocco) belonged to the same cluster. The other *E. cloacae* isolates were distinct (data not shown).

Genetic location the *bla*$_{OXA-48}$ gene

Using the specific primers designed from the reference plasmid pOXA-48a of *K. pneumoniae* 11978 [27] to amplify its replicase gene, 99 of the 107 isolates (92.5%) carried an IncL/M-pOXA-48a-like backbone. For the eight other isolates (*P. rettgeri* isolate no. 15, *E. coli* isolates no. 19 to 24, and *E. coli* isolate no. 37), mating-out assays were performed and transconjugants harbouring the *bla*$_{OXA-48}$ gene were obtained for *P. rettgeri* isolate no. 15 and *E. coli* isolate no. 37. Plasmid DNA analysis of the two *E. coli* transconjugants revealed a single plasmid. Those two *bla*$_{OXA-48}$-positive plasmids corresponded to a ca. 150 kb IncA/C-type plasmid identified from *P. rettgeri* isolate no. 15 from Turkey and a ca. 160-kb IncF-type plasmid from an *E. coli* isolate from France. Despite repeated attempts, transconjugants or transformants were not obtained for six of the seven *E. coli* isolates belonging to ST38. Interestingly, I-*Ceu*I analysis confirmed the chromosomal location of the *bla*$_{OXA-48}$ gene in those six isolates (data not shown). Furthermore, one out of the seven *bla*$_{OXA-48}$-positive ST38 *E. coli* harboured the epidemic OXA-48 IncL/M-type plasmid.

Genetic environment of the *bla*$_{OXA-48}$ gene

The *bla*$_{OXA-48}$ gene was flanked by two copies of IS*1999*. In 21 isolates (19.6%), the upstream copy remained intact. This structure corresponded to transposon Tn*1999*, whereas 84 isolates (78.5%) had a Tn*1999.2* transposon structure in which the IS*1999* is disrupted by insertion of an IS1R element [4]. In two isolates

Figure 1

Sequence types represented among OXA-48-producing *Klebsiella pneumoniae* isolates, 2001–11 (n=67)

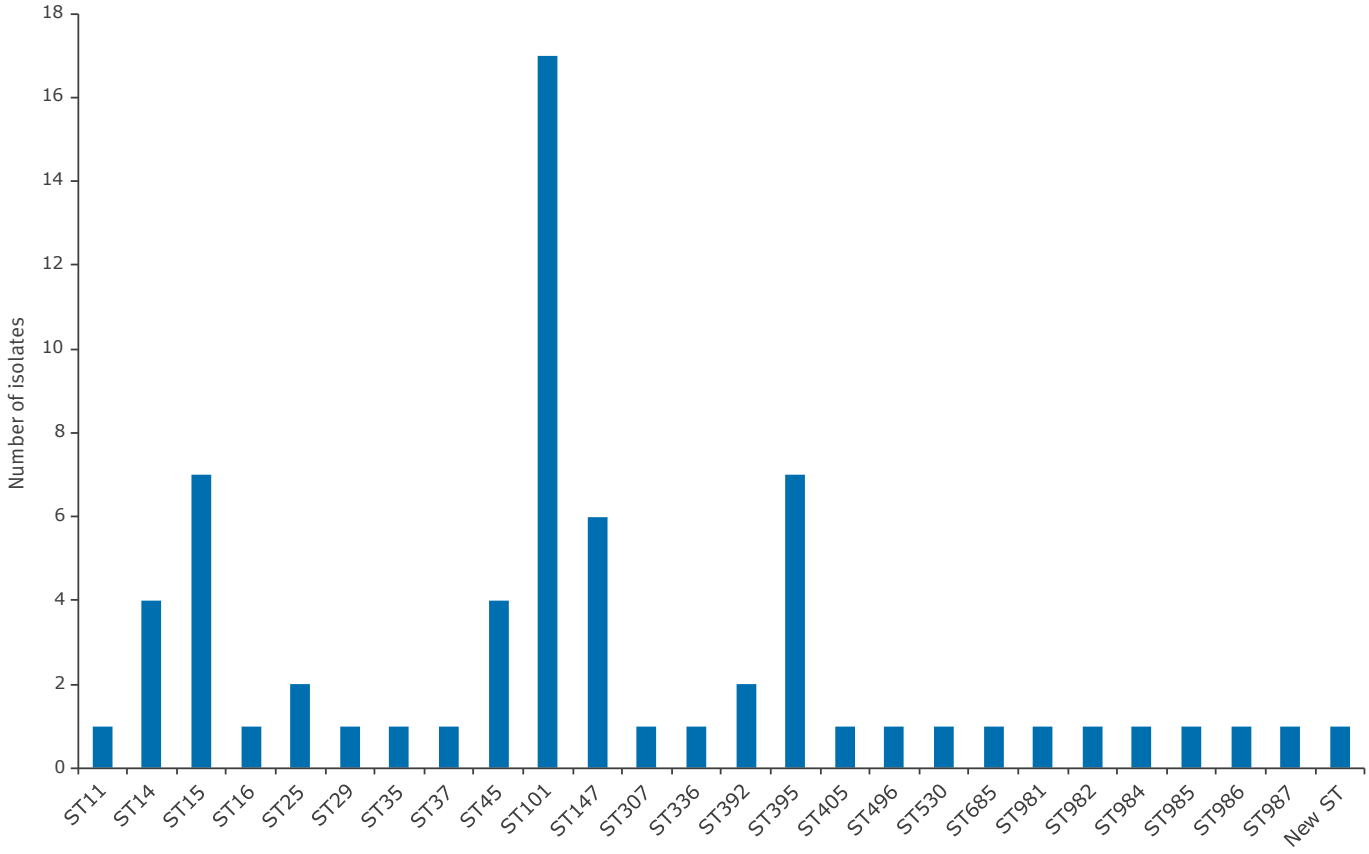
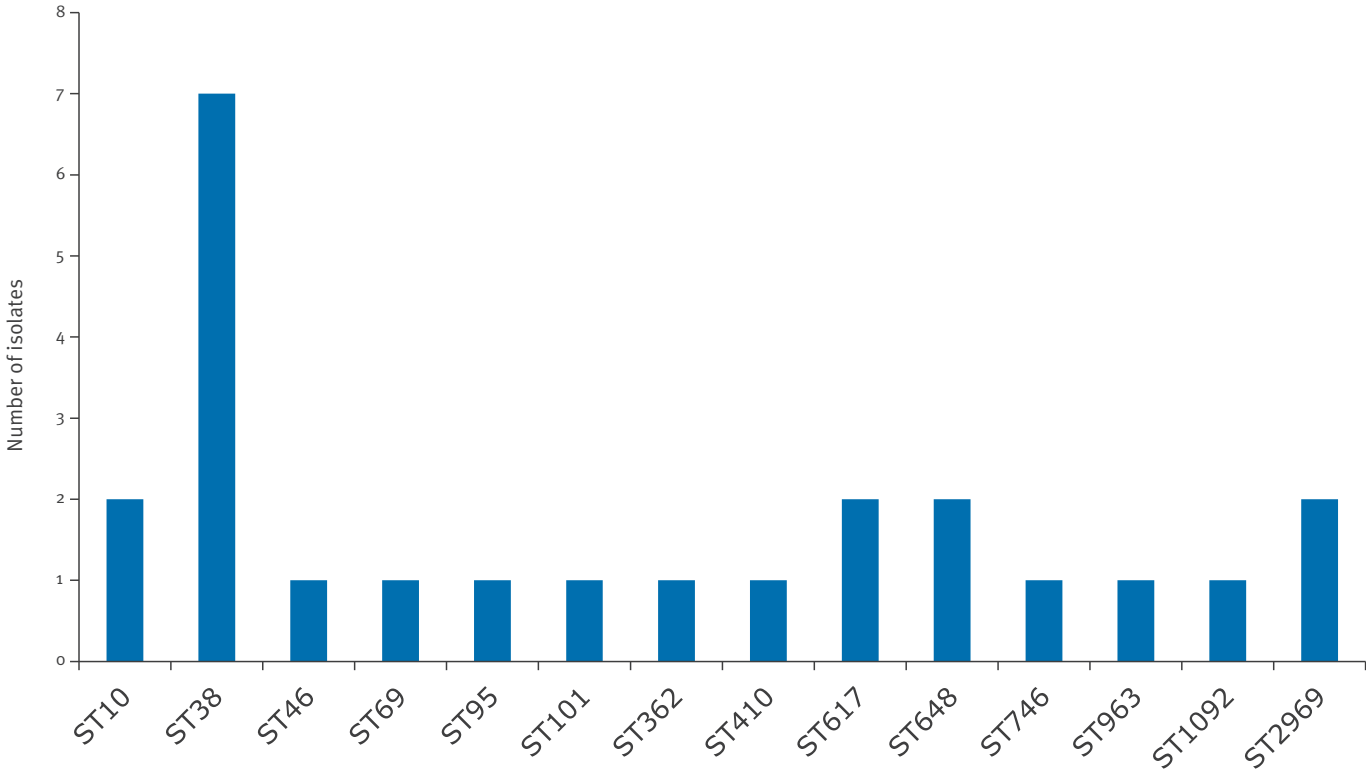


Figure 2

Sequence types represented among OXA-48-producing *Escherichia coli* isolates, 2001–11 (n=24)



(*E. cloacae* isolate no. 4 and *E. coli* isolate no. 17, recovered from the same patient), a new Tn*1999* derivative was identified. This new transposon Tn*1999.4* was composed of Tn*1999.2* disrupted by another transposon, Tn*2015* which, in turn, was composed of IS*Ecp1*, $bla_{CTX\text{-}M\text{-}15}$ and a truncated Tn*2* transposase [32].

Discussion

We have analysed here many different features of 107 known OXA-48-positive enterobacterial isolates which are widely distributed at least in several European and North African countries, and also in Turkey. Noticeably, 25% of the OXA-48 beta-lactamase producers remained susceptible to broad-spectrum cephalosporins, which therefore present possible therapeutic options. At least positive therapeutic outcomes have been obtained using an animal model of infection and broad-spectrum cephalosporins [33]. Those ESBL-negative isolates were most often susceptible to the other classes of antibiotics, which is in line with the fact that the epidemic plasmid encoding the $bla_{OXA\text{-}48}$ gene does not carry additional resistance determinants [28].

However, 75% of the OXA-48-positive isolates in our study harboured an additional ESBL-encoding gene that confers resistance to broad-spectrum cephalosporins. We have recently reported the genetic association of the $bla_{CTX\text{-}M\text{-}15}$ and $bla_{OXA\text{-}48}$ genes on the same transposon, indicating that this combination of multidrug-resistance genes may spread further in the future [32]. In addition, most of those ESBL-producing isolates were resistant to non-beta-lactam antibiotics, due to other resistance mechanisms. It is worth mentioning that 70 isolates (65%) were susceptible to imipenem and meropenem according to CLSI guidelines, further complicating the detection of OXA-48-producing isolates in laboratories. Conversely, most isolates showed intermediate susceptibility or resistance to ertapenem. Ertapenem may thus be the most appropriate carbapenem molecule for detecting OXA-48 producers. Therefore, a selective medium containing ertapenem has recently been developed for the detection of all types of carbapenemase producers including the OXA-48 beta-lactamase producers [34]. Taking into account the fact that 75% of the OXA-48 isolates were ESBL producers and the level of resistance to non-beta-lactam molecules, treatment options for infections caused by OXA-48 beta-lactamase producers may be limited. The efficacy of carbapenems in treating infections due to OXA-48 beta-lactamase producers with susceptibility or low-level resistance to several carbapenems remains debatable, because carbapenems have been shown to be an inefficient therapy for treating mice with induced peritonitis caused by an OXA-48-producing *K. pneumoniae* [33]. Also, imipenem-containing therapy failed to treat several OXA-48 infections in humans [4,11]. A single report described imipenem as efficient treatment against bacteraemia due to an OXA-48 *K. pneumoniae* isolate [35]. Controlled trials are needed to evaluate the real clinical efficacy of carbapenems in treating infections due to OXA-48 beta-lactamase producers.

The clonal distribution of OXA-48 beta-lactamase-positive isolates is interesting because a quarter of the *K. pneumoniae* isolates belonged to ST101. OXA-48-positive *K. pneumoniae* isolates belonging to ST101 have recently been implicated in an outbreak in Spain, and have also been detected in Tunisia [11,12]. We report here that the ST101 isolates were recovered from Tunisia, Morocco, and from South Africa and France from patients who did not travel abroad, suggesting that this ST has now widely spread in European countries and in Africa. Seven *K. pneumoniae* isolates belonged to ST395, a ST implicated in clonal outbreaks in Europe [11,14]. Interestingly, we detected seven ST15 among *K. pneumoniae* isolates recovered from patients who had a link with Morocco. That sequence type corresponds to an internationally occurring clone and has been associated with different ESBL genes, but also with the metallo-beta-lactamase genes coding for NDM and VIM [36,37]. The occurrence of OXA-48 beta-lactamase in a ST15 *K. pneumoniae* isolate had been reported only once, in 2012, in an isolate from Finland [38]. Those data are likely to indicate that a novel OXA-48 *K. pneumoniae* clone belonging to ST15 may emerge in Morocco. *K. pneumoniae* isolates belonging either to ST392 or ST147 (differing at a single locus) were identified in a total of eight isolates, with the two ST392 collected in Morocco and the six ST147 collected in Belgium, Turkey and France, and also from patients originating from Tunisia or Libya. This result highlights the dissemination of another OXA-48-producing clone, mainly in the Mediterranean area. The other *K. pneumoniae* isolates belonged to diverse ST, supporting the hypothesis of the widespread dissemination of a single $bla_{OXA\text{-}48}$-positive IncL/M plasmid among various genetic backgrounds. Overall, there is no association between ST type and ESBL type among OXA-48 producers.

Among the 24 *E. coli* isolates, seven were of ST38, showing that this clone is widely disseminated, as previously suggested [7,39]. Interestingly, the $bla_{OXA\text{-}48}$ gene was chromosomally located in six of those isolates, as was speculated for the ST38 *E. coli* isolates recovered in the United Kingdom [7]. Such chromosomal location of the $bla_{OXA\text{-}48}$ gene in *E. coli* may be associated to a lower level of resistance (a single gene copy). The other 17 *E. coli* isolates were genetically distinct. Furthermore, it is interesting to note that 16 of the 24 OXA-48-positive *E. coli* belonged to phylogenetic group D or B2, which mainly include virulent strains. The *E. cloacae* isolates were overall clonally diverse.

As suggested previously, the $bla_{OXA\text{-}48}$ gene was located on a 62 kb IncL/M plasmid in most of our isolates (n=99, 92.5%), indicating that current spread of OXA-48 beta-lactamase producers is mainly related to the diffusion of this plasmid. The dissemination of the $bla_{OXA\text{-}48}$ gene is also associated with the spread of different clones.

Interestingly, 20% of OXA-48-producing isolates collected in France were considered to be autochthonous, indicating that the *bla*$_{OXA-48}$ gene has already spread in the community in France. This latter result indicates ongoing diffusion of OXA-48-type genes in Europe.

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