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Phylogenetic lineages, clones and β-lactamases in an international collection of *Klebsiella oxytoca* isolates non-susceptible to expanded-spectrum cephalosporins

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Objectives: The objective of this study was to examine *Klebsiella oxytoca* clonal and phylogenetic diversity, based on an international collection of carriage isolates non-susceptible to expanded-spectrum cephalosporins (ESCs).

Methods: The study material comprised 68 rectal carriage *K. oxytoca* isolates non-susceptible to ESCs recovered in 2008–11 from patients in 14 hospitals across Europe and Israel. ESC resistance was tested phenotypically; genes encoding ESBLs, AmpC cephalosporinases and carbapenemases were amplified and sequenced. The isolates were typed by PFGE and MLST, followed by sequencing of bla_{OXY} genes.

Results: MLST and PFGE distinguished 34 STs and 47 pulsotypes among the isolates, respectively. Six STs were split into several pulsotypes each. Five STs were more prevalent (n=2–9) and occurred in several countries each, including ST2, ST9 and ST141, which belong to a growing international clonal complex (CC), CC2. Four phylogenetic lineages were distinguished, each with another type of chromosomal OXY-type β-lactamase. Three of these, with OXY-1/-5, OXY-2 types and OXY-4, corresponded to previously described phylogroups KoI, KoII and KoIV, respectively. A single isolate from Israel represented a distinct lineage with a newly defined OXY-7 type. The phylogroups showed interesting differences in mechanisms of ESC resistance; KoI strains rarely overexpressed the OXY enzymes but commonly produced ESBLs, whereas KoII strains often were OXY hyperproducers and carried ESBLs much less frequently. AmpCs (DHA-1) and carbapenemases (VIM-1) occurred sporadically.

Conclusions: The study confirmed the high genetic diversity of the collection of *K. oxytoca* ESC-non-susceptible isolates, composed of phylogroups with distinct types of OXY-type β-lactamases, and revealed some STs of broad geographical distribution.

Introduction

Klebsiella oxytoca is an opportunistic pathogen causing nosocomial infections, often in ICU patients or those under immunosuppressive therapy,[1] including bloody diarrhoea after antibiotic treatment.[2] It is naturally resistant to amino- and carboxypenicillins owing to low-level production of chromosomal β-lactamases of the OXY group.[3–5] Overproduction of these due to promoter-up mutations results in reduced susceptibility or resistance to other β-lactams, such as penicillin–inhibitor combinations, cefuroxime, cefotaxime and aztreonam, and is observed in 10%–20% of clinical isolates.[5–8] Fevre *et al.*[9] described six lineages of OXY-type β-lactamases, OXY-1 to -6, that evolved over ~100 million years in parallel to housekeeping genes, as OXY gene phylogeny is congruent with the phylogeny based on genes *gyrA*, *rpoB* and *gapDH*. In addition, *K. oxytoca* can acquire other enzymes hydrolysing newer-generation β-lactams, like ESBLs, AmpC-type cephalosporinases or carbapenemases, and outbreaks caused by such strains have been reported.[10–13]

The first *Klebsiella* MLST scheme was introduced for *Klebsiella pneumoniae* in the mid-2000s, revolutionizing population studies of this pathogen.[14] In May 2014 this scheme, including the *rpoB* gene used also as a species phylogenetic marker, was adapted to *K. oxytoca* by Herzog *et al.*,[15] opening the way to similar

investigations. The first analysis carried out on isolates mainly from Austria yielded 44 STs.[15]

Here, we report a multinational MLST study of *K. oxytoca*, using isolates that were non-susceptible to expanded-spectrum cephalosporins (ESCs), collected during the EU-funded project MOSAR.[16] The MLST data were compared with PFGE and OXY-type β-lactamase sequences, and supplemented by the profiles of acquired ESC-hydrolysing β-lactamases.

Materials and methods

Study design, clinical isolates and their phenotypic analysis

Between mid-2008 and mid-2011, 17 945 patients in 18 ICUs and rehabilitation units across Europe and Israel were screened for colonization with ESC-resistant Enterobacteriaceae. Rectal swabs were collected at admission, then regularly during hospitalization, and at discharge. The swabs were plated onto Brilliance™ ESBL Agar (Oxoid, Basingstoke, UK); Enterobacteriaceae colonies were identified using the manufacturer's instructions. One colony of each morphotype was collected for definite analysis. Species identification was performed using the Vitek 2 system (bioMérieux, Marcy-l'Étoile, France). All isolates were tested for ESBL and AmpC expression by the ESBL double-disc synergy test (DDST) in the absence and presence of cloxacillin (250 mg/L),[17] and for susceptibility to ertapenem, imipenem and meropenem according to EUCAST (http://eucast.org). Isolates non-susceptible to at least one carbapenem were subjected to metallo-β-lactamase (MBL), KPC and OXA-48 detection, using DDST with EDTA,[18] the phenylboronic acid disc test[19] and a temocillin disc,[20] respectively.

Typing

PFGE was performed as described previously,[21] with the use of the XbaI restriction enzyme (Fermentas, Vilnius, Lithuania). PFGE types and subtypes were discerned according to Tenover *et al.*[22] Subsequently, electrophoretic patterns were compared by the BioNumerics Fingerprinting software (Version 6.01, Applied Maths, Sint-Martens-Latem, Belgium), using the Dice coefficient and the UPGMA clustering method, with 1% tolerance in band position differences. MLST was carried out according to Herzog *et al.*;[15] the database available at http://pubmlst.org/koxytoca was used for assigning STs. The relatedness between STs was analysed by eBURST (http://eburst.mlst.net). The clonal diversity index and CIs were calculated according to Grundmann *et al.*[23] Nucleotide diversity was calculated using DnaSP Ver. 5.10.01.[24] Phylogenetic analysis of nucleotide sequences was performed using Lasergene MegAlign software (DNASTAR Inc., Madison, Wisconsin, USA), using the CLUSTAL W alignment algorithm. Searches of the GenBank database were carried out using the NCBI BLASTn option (www.ncbi.nlm.nih.gov).

Analysis of OXY-type β-lactamases

PCR and sequencing of bla_{OXY}-like genes with promoters were performed as described previously.[9] Amino acid sequences of OXY-type β-lactamases were analysed against the nomenclature database of OXY variants available at http://bigsdb.web.pasteur.fr/klebsiella.

Analysis of acquired β-lactamases

Identification of $bla_{CTX\text{-}M}$-, bla_{SHV}-, bla_{TEM}-, bla_{DHA}- and bla_{VIM}-like genes was done by PCRs and sequencing as reported previously.[25–28]

Nucleotide sequence accession numbers

Nucleotide sequences of $bla_{OXY\text{-}1\text{-}8}$, $bla_{OXY\text{-}1\text{-}9}$, $bla_{OXY\text{-}2\text{-}16}$, $bla_{OXY\text{-}2\text{-}17}$, $bla_{OXY\text{-}2\text{-}18}$, $bla_{OXY\text{-}2\text{-}19}$, $bla_{OXY\text{-}2\text{-}20}$, $bla_{OXY\text{-}5\text{-}3}$ and $bla_{OXY\text{-}7\text{-}1}$ were assigned the GenBank database accession numbers KT001246–KT001254, respectively. Novel OXY variants were deposited in the OXY β-lactamase nomenclature database at http://bigsdb.web.pasteur.fr/klebsiella.

Results and discussion

Clonality of the K. oxytoca isolates

Sixty-eight ESC-non-susceptible patient-unique *K. oxytoca* isolates were identified during the MOSAR study. These were recovered in variable numbers (n=1–20) in 14 centres from eight countries (Table 1). PFGE revealed 61 different patterns clustered into 47 pulsotypes, each characteristic of one centre (Figure S1, available as Supplementary data at *JAC* Online). MLST classified the isolates into 34 STs, of which 24 were new: 137, 141–144, 147–163, 165 and 167. PFGE was more discriminatory, splitting six STs into several pulsotypes each, and so being a good tool for *K. oxytoca* outbreak investigations. The MLST-based clonal diversity index was 94.3% (CI 91.6%–97.0%), reflecting remarkable genetic variety of the isolates. The average nucleotide diversity per MLST gene within the sample, π, ranged from 1.1% to 5.2% (Table S1), being similarly high as previously (1.3%–5.9%).[15] Concatemers of the MLST alleles of the 34 STs were subjected to phylogenetic analysis. The phylogenetic tree comprised four lineages of related STs, designated A–D (Figure 1 and Table S2). The lineages A and D dominated, with 17 STs and 23 isolates and 14 STs and 42 isolates, respectively.

Eight STs had more than one isolate (n=42; 61.8%), including five STs present in more than one hospital and country, and split into two to six pulsotypes each (Table 1). These 'major' STs belonged to the predominant lineages and were: ST9 (n=9), ST2 (n=8), ST88 (n=6), ST141 (n=3) and ST36 (n=2). Their contribution to larger local *K. oxytoca* groups varied from the vast majority in the Spanish rehabilitation unit GI (n=13; 86.7%) to a clear minority in the Latvian ICU RI (n=4; 20.0%). Twenty-nine STs were observed in single hospitals, including the most prevalent, ST153 (n=10), which occurred only in Latvia and consisted of a single pulsotype. Ten STs have been identified also in other studies, e.g. ST2 (isolates from Austria and Spain), ST9 (Austria and Germany) and ST36 (Austria)[15] (http://pubmlst.org/koxytoca).

The eBURST analysis was performed on the entire *K. oxytoca* MLST database (http://pubmlst.org/koxytoca), containing 161 STs as of 7 May 2015 (Figure 2). Of the 34 STs found in this work, 12 were clustered into five eBURST groups of ≥3 STs each (Table 1). The largest one was a clonal complex (CC) with ST2 as the central genotype, distinguished previously and designated as CC2.[15] In the study sample it comprised ST2, ST9, ST141, ST154 and ST155, with 22 isolates (32.4%) from nine hospitals (France, Spain, Portugal, Italy, Latvia and Israel). According to Herzog *et al.*[15] and the database, CC2 contains six more STs of broad distribution, including Austria, the Netherlands and China. The high prevalence and wide geographical spread of CC2 and its several individual STs may indicate their global character, similar to some CCs and STs of *K. pneumoniae*, *Escherichia coli* and *Enterobacter cloacae*.[29–33] The four other eBURST groups consisted of three STs each. The first of these included ST4, originally identified in Austria[15] and found here in Latvia, ST5 from Austria[15] and ST161 from Greece in this study. This group, consisting only of ST4 and ST5, was previously defined as 'CC8'.[15] ST144 of an

Table 1. *K. oxytoca* eBURST groups[a] and singleton STs identified in the study; OXY β-lactamase variants, sequences of bla_{OXY} gene promoter −10 hexamers, geographical distribution, prevalence, pulsotypes and acquired ESC-hydrolysing β-lactamases

Clonal status[a,b,c]	ST[d]	Phylogenetic lineage	OXY variant[d]	−10 box (G/A)GATAGT[e]	Centre (country)	No. of isolates (% of all isolates)[f]	No. of pulsotypes (subtypes=patterns)	ESBL, AmpC or carbapenemase types (*n*)
CC2 (STs: 2, 9, 18, 19, 57, 58, 61, 63, 141, 154, 155)	ST2	D	**OXY-2-16**	(A)**T**ATAGT	FS (Italy)	1	1	
					RI (Latvia)	1	1	
				(A)GATA**A**T	GI (Spain)	1	1	SHV-12
					PO (Portugal)	1	1	
					RI (Latvia)	3	1 (2)	CTX-M-15 (3)
					TA (Israel)	1	1	SHV-2
	ST9	D	OXY-2-5	(A)GATA**A**T	GI (Spain)	6	1 (4)	
				(A)GATAGT	BM (France)	2	1 (2)	SHV-12 (2)
					BA (Spain)	1	1	CTX-M-3
	ST141	D	OXY-2-7	(A)**T**ATAGT	GI (Spain)	1	1	
					VR (Portugal)	1	1	
			OXY-2-14	(A)**T**ATAGT	BM (France)	1	1	
	ST154	D	**OXY-2-19**	(A)**T**ATAGT	HM (France)	1	1	
	ST155	D	OXY-2-8	(A)GATA**A**T	FS (Italy)	1	1	
						total 22 (32.3%)		
sglt	ST36	D	OXY-2-11	(A)**T**ATAGT	SJ (France)	1	1	
				(A)GATAGT	TA (Israel)	1	1	SHV-5
SLV of ST112	**ST152**	D	**OXY-2-17**	(A)GATAGT	BM (France)	1	1	DHA-1
SLV of ST1	**ST153**	D	**OXY-2-18**	(A)GATAGT	RI (Latvia)	10	1 (8)	CTX-M-15; SHV-12 (2) SHV-12 (4) CTX-M-15 CTX-M-3 (3)
sglt	**ST156**	D	OXY-2-1	(A)GATA**A**T	BM (France)	1	1	
ST5-ST4-ST161	ST4	D	OXY-2-2	(A)GATAGT	RI (Latvia)	1	1	TEM-52
	ST161	D	OXY-2-1	(A)GATA**A**T	LA (Greece)	1	1	
ST53-ST30-ST150	**ST150**	D	OXY-2-5	(A)**T**ATAGT	AT (Greece)	1	1	
SLV of ST65	**ST159**	D	OXY-2-7	(A)GATA**A**T	BM (France)	2	1	
SLV of ST96	**ST147**	D	**OXY-2-20**	(A)GATA**A**T	LU (Luxembourg)	1	1	
sglt	**ST167**	A	OXY-1-1	(G)GATAGT	LH (Israel)	1	1	CTX-M-15
sglt	**ST142**	A	OXY-1-1	(G)GATAGT	TA (Israel)	1	1	CTX-M-39
ST43-ST144-ST108	**ST144**	A	OXY-1-1	(G)GATAGT	TA (Israel)	1	1	SHV-5
SLV of ST146	**ST148**	A	OXY-1-1	(G)GATAGT	LU (Luxembourg)	1	1	CTX-M-15
sglt	**ST151**	A	OXY-1-1	(G)GATAGT	HM (France)	1	1	TEM-3
ST149-ST135-ST158	ST135	A	OXY-1-2	(G)GATAGT	HM (France)	1	1	SHV-12
	ST149	A	OXY-1-2	(G)GATAGT	LU (Luxembourg)	1	1	SHV-12

	ST158	A	OXY-1-2	(G)GATA**A**T	SJ (France)	1	1	TEM-24
sglt	ST98	A	OXY-1-3	(G)GATAGT	RI (Latvia)	1	1	SHV-12
sglt	ST88	A	**OXY-1-8**	(G)GATAGT	GI (Spain)	5	1 (4)	CTX-M-9 (5)
					HM (France)	1	1	SHV-4
sglt	**ST143**	A	**OXY-1-8**	(G)GATAGT	TA (Israel)	1	1	SHV-5
sglt	**ST157**	A	**OXY-1-9**	(G)GATAGT	TA (Israel)	1	1	SHV-12
sglt	ST66	A	**OXY-5-3**	(G)GATAGT	RI (Latvia)	1	1	SHV-2a
sglt	ST82	A	OXY-5-2	(G)GATAGT	RI (Latvia)	1	1	SHV-2a
sglt	ST86	A	**OXY-5-3**	(G)GATAGT	RI (Latvia)	1	1	CTX-M-15
sglt	**ST137**[g]	A	OXY-5-1	(G)GATAGT	GI (Spain)	2	2	VIM-1 (2)
sglt	**ST162**	A	OXY-5-1	(G)GATAGT	LH (Israel)	1	1	SHV-12
SLV of ST81	**ST160**	B	OXY-4-1	(G)GATAGT	TA (Israel)	1	1	CTX-M-15
sglt	**ST163**	B	OXY-4-1	(G)GATAGT	RI (Latvia)	1	1	CTX-M-15
sglt	**ST165**	D	**OXY-7-1**	(G)GATAGT	TA (Israel)	1	1	CTX-M-15; TEM-33[h]

[a]eBURST groups comprise CC2, groups of three closely related STs for which no central genotypes may be distinguished, and SLV pairs.
[b]eBURST groups and singleton (sglt) STs have been ordered arbitrarily according to their classification to phylogenetic lineages (Figure 1).
[c]In parentheses are shown all STs forming a given eBURST group, including the STs not identified in this study, but submitted by others to the database (http://pubmlst.org/koxytoca; as of 7 May 2015).
[d]STs and OXY enzymes identified in the study; numbers in bold indicate new STs and OXY variants.
[e]Consensus sequence of the −10 hexamer in *K. oxytoca*.
[f]Percentages among all study isolates are shown in parentheses only for the entire CC2.
[g]These two VIM-1-producing ST137 isolates were reported previously.[36]
[h]TEM-33 is an inhibitor-resistant TEM β-lactamase (IRT-5) (http://www.lahey.org/Studies/).

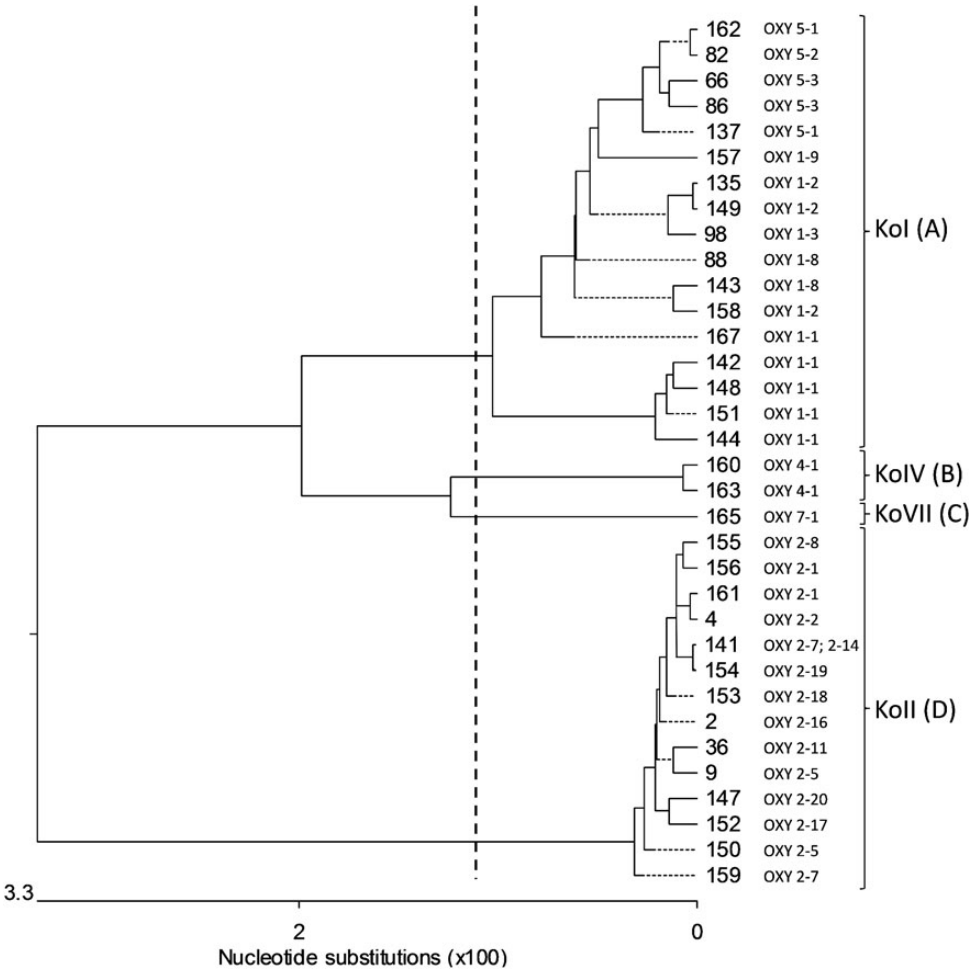

Figure 1. Unrooted UPGMA phylogenetic tree of the 34 STs identified in the study, based on concatemers of the MLST loci. The tree was constructed using the Lasergene MegAlign software (DNASTAR Inc.). Only one isolate per ST was included in the dataset. Numbers on the right correspond to STs, followed by variants of OXY β-lactamases detected in individual STs. Lineages are shown on the right. The broken vertical line indicates the cluster cut-off value used, which was 115 nucleotide substitutions per concatemer (3003 bp).

isolate from Israel was a single-locus variant (SLV) of ST43 and ST108 from Austria,[15] thus forming a distinct group. ST150 from Greece was clustered with ST30 and ST53 from the USA (http://pubmlst.org/koxytoca). Finally, ST135, ST149 and ST158 from France and Luxembourg formed a group with ST135 at the central position, submitted first from Austria (http://pubmlst.org/koxytoca). Like other clusters consisting of STs not found in this study (Figure 2), these groups may be upgraded to CCs when more clonality data accumulate.

The eBURST results were analysed against the phylogenetic tree of the sample (Figure 1) and of the entire *K. oxytoca* MLST database (data not shown). All STs of one eBURST group were located also in the same lineage. Numbers of polymorphisms in the alleles differentiating all SLVs within CC2 and other groups were low ($n=1-5$; usually 1–2) in the context of the significant nucleotide diversity within the sample (Tables S1 and S2). These results suggest a higher contribution of mutational evolution within eBURST groups, as homologous recombination among distant genotypes could introduce more nucleotide differences.

OXY-like β-lactamases

Of the 22 variants of OXY-like β-lactamases found in the study isolates (Table 1), 21 enzymes were of four of the six types distinguished previously, namely OXY-1, -2, -4 and -5.[9] The remaining ST165 isolate from Israel had an enzyme that differed remarkably from all those in the database (http://bigsdb.web.pasteur.fr/klebsiella), and was classified as the first variant of a new type, OXY-7-1 (Figure S2). The most prevalent type was OXY-2, with 12 variants in 42 isolates (61.8%) of 15 STs, followed by OXY-1, with 5 variants in 17 isolates (25.0%) of 12 STs. Apart from OXY-7-1, eight other variants were new. The high contribution of the new variants to the entire database ($n=37$; as of 7 May 2015) suggests that the actual number of OXY types and variants has been underestimated and that additional diversity will be revealed by analysing more *K. oxytoca* isolates.

The OXY β-lactamases correlated well with STs; in all cases but one the STs with more than one isolate were homogeneous in OXY variants. The only exception was ST141, which had either OXY-2-7 (in Spain and Portugal) or OXY-2-14 (France), differing by one amino acid substitution. The analysis of the OXY amino acid

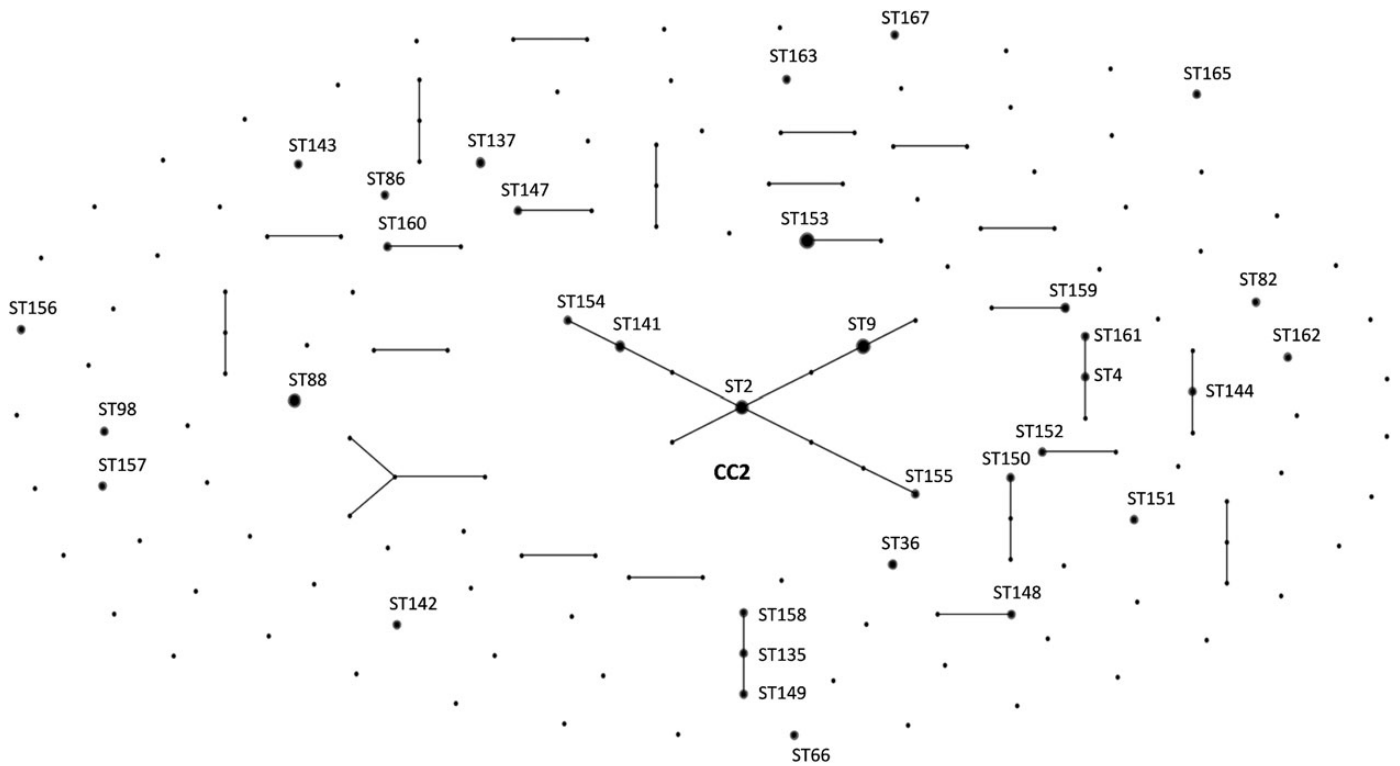

Figure 2. Population structure of the ESC-non-susceptible *K. oxytoca* isolates identified in the study, shown in the context of all 161 of the STs present in the global MLST database (http://pubmlst.org/koxytoca; as of 7 May 2015). The scheme was constructed using eBURST analysis. STs are symbolized by dots; the STs identified in the study are represented by dots in circles and given ST numbers. The size of a dot is related to the number of isolates belonging to the corresponding ST. SLVs are linked by continuous lines.

sequences against the MLST phylogenetic tree of the sample (Figure 1) revealed good correlation between OXY types and phylogenetic lineages, with types OXY-1 and OXY-5 corresponding to the lineage A and OXY-4, OXY-7 and OXY-2 being found only in lineages B, C and D, respectively. This observation was consistent with the earlier phylogenetic *K. oxytoca* studies, based on one to three housekeeping genes and OXY-like β-lactamases.[9,34,35] According to the nomenclature used by Fevre *et al.*,[9] our lineages A, B and D corresponded to phylogroups KoI, KoIV and KoII, respectively, whereas lineage C with OXY-7 represents a new branch KoVII, with KoIV as a sister group.

Sequencing of bla_{OXY} gene promoters revealed promoter-up mutations in 27 isolates (38.2%) of 13 STs. Most of these ($n=26$) had bla_{OXY-2}-type genes (lineage KoII); the only exception was an isolate with the bla_{OXY-1}-type gene (KoI) (Table 1). The mutations were common among the 'major' KoII STs, being observed in 8/8 ST2, 6/9 ST9, 1/2 ST36 and 3/3 ST141 isolates. None of the acquired β-lactamase genes tested was identified in 21 of these isolates, suggesting that OXY overproduction was the main mechanism of ESC resistance.[5–8] Concordantly with previous studies,[7] the mutations occurred in the −10 hexamer, being the G to A transition (at position 5) in 19 isolates or G to T transversion (position 1) in eight isolates. This study confirmed the significant contribution of OXY overexpression to ESC resistance in *K. oxytoca*, especially in OXY-2 producers.[5–8] Being the first analysis with the use of MLST, it showed that it may occur in any ST of the OXY-2-producing lineage KoII.

Acquired ESC-hydrolysing β-lactamases

Forty-seven isolates (69.1%) produced acquired ESC-hydrolysing β-lactamases, including 44 ESBL (64.7%) producers, two MBL producers and one AmpC producer (Table 1). ESBLs occurred in 24 STs of all lineages; however, interesting differences were observed between KoI and KoII. Among the KoI isolates, ESBL producers vastly predominated ($n=21$; 91.3%) and represented all of their 17 STs. This contrasted strongly with KoII, where only a half of isolates ($n=20$; 47.6%), belonging to only 5 of 14 STs, were ESBL-positive. CTX-M- and SHV-like enzymes occurred in 22 and 21 isolates, respectively (50.0% and 47.7% of ESBL-positive isolates). CTX-M-15 and SHV-12 were the most frequent ESBLs, each expressed by 12 isolates (27.3%) of eight STs. Each of the 'major' clones ST2, ST9 and ST88 expressed multiple ESBL types, correlating with site of isolation and pulsotype. Therefore, similar to other species,[29,33] the wide spread *K. oxytoca* STs are not strictly associated with a single ESBL type.

Two ST137 isolates of different pulsotypes from Spain were the only carbapenem-non-susceptible isolates, produced the MBL VIM-1 and were included in a report on MBL producers from the MOSAR project.[36] Only one ST152 isolate from France had an AmpC-type cephalosporinase DHA-1.

Conclusions

This is the first study, to our knowledge, on ESC-non-susceptible *K. oxytoca* that combines MLST-based phylogenetic and clonal

analysis with β-lactamase profiling. It revealed high genetic diversity of the isolates colonizing patients in clinical sites across Europe and Israel. Together with other works[15] (http://pubmlst.org/koxytoca), our study showed a broad international distribution of several STs and clonal clusters, especially CC2, contributing largely to the entire collection. The results were congruent with previous phylogenetic analyses that showed several *K. oxytoca* clades associated with OXY-like β-lactamase types.[9,34,35] A new OXY type, OXY-7, was identified within a distinct lineage. The predominance of two major lineages, KoI (OXY-1/-5) and KoII (OXY-2), was confirmed.[9,34,35] Interestingly, organisms of these phylogroups differed remarkably in their main mechanisms of ESC resistance: whereas KoI strains readily acquired ESBLs and rarely overexpressed OXYs, the KoII ones were mainly OXY hyperproducers and carried ESBLs less frequently. Further studies on the ecology and microbiology of *K. oxytoca* lineages are necessary to verify and explain this observation, especially because this study had several limitations associated with the MOSAR study design regarding the isolates' collection. The isolates were recovered from rectal carriage only and from hospitalized patients only, and were preselected based on their non-susceptibility to ESCs. These limitations must be considered while interpreting the data presented, mainly those on the occurrence and distribution of individual clones.

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Transparency declarations

None to declare.

Supplementary data

Figures S1 and S2 and Tables S1 and S2 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

References

1 Podschun R, Ullmann U. *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin Microbiol Rev* 1998; **11**: 589–603.

2 Hogenauer C, Langner C, Beubler E *et al*. *Klebsiella oxytoca* as a causative organism of antibiotic-associated hemorrhagic colitis. *N Engl J Med* 2006; **355**: 2418–26.

3 Arakawa Y, Ohta M, Kido N *et al*. Chromosomal β-lactamase of *Klebsiella oxytoca*, a new class A enzyme that hydrolyzes broad-spectrum β-lactam antibiotics. *Antimicrob Agents Chemother* 1989; **33**: 63–70.

4 Fournier B, Roy PH, Lagrange PH *et al*. Chromosomal β-lactamase genes of *Klebsiella oxytoca* are divided into two main groups, bla_{OXY-1} and bla_{OXY-2}. *Antimicrob Agents Chemother* 1996; **40**: 454–9.

5 Livermore DM. β-Lactamases in laboratory and clinical resistance. *Clin Microbiol Rev* 1995; **8**: 557–84.

6 Fournier B, Arlet G, Lagrange PH *et al*. *Klebsiella oxytoca*: resistance to aztreonam by overproduction of the chromosomally encoded β-lactamase. *FEMS Microbiol Lett* 1994; **116**: 31–6.

7 Fournier B, Lagrange PH, Philippon A. β-Lactamase gene promoters of 71 clinical strains of *Klebsiella oxytoca*. *Antimicrob Agents Chemother* 1996; **40**: 460–3.

8 Fournier B, Gravel A, Hooper DC *et al*. Strength and regulation of the different promoters for chromosomal β-lactamases of *Klebsiella oxytoca*. *Antimicrob Agents Chemother* 1999; **43**: 850–5.

9 Fevre C, Jbel M, Passet V *et al*. Six groups of the OXY β-lactamase evolved over millions of years in *Klebsiella oxytoca*. *Antimicrob Agents Chemother* 2005; **49**: 3453–62.

10 Lowe C, Willey B, O'Shaughnessy A *et al*. Outbreak of extended-spectrum β-lactamase-producing *Klebsiella oxytoca* infections associated with contaminated handwashing sinks. *Emerg Infect Dis* 2012; **18**: 1242–7.

11 Decre D, Burghoffer B, Gautier V *et al*. Outbreak of multi-resistant *Klebsiella oxytoca* involving strains with extended-spectrum β-lactamases and strains with extended-spectrum activity of the chromosomal β-lactamase. *J Antimicrob Chemother* 2004; **54**: 881–8.

12 Vergara-Lopez S, Dominguez MC, Conejo MC *et al*. Wastewater drainage system as an occult reservoir in a protracted clonal outbreak due to metallo-β-lactamase-producing *Klebsiella oxytoca*. *Clin Microbiol Infect* 2013; **19**: E490–8.

13 Hoenigl M, Valentin T, Zarfel G *et al*. Nosocomial outbreak of *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella oxytoca* in Austria. *Antimicrob Agents Chemother* 2012; **56**: 2158–61.

14 Diancourt L, Passet V, Verhoef J *et al*. Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. *J Clin Microbiol* 2005; **43**: 4178–82.

15 Herzog KA, Schneditz G, Leitner E *et al*. Genotypes of *Klebsiella oxytoca* isolates from patients with nosocomial pneumonia are distinct from those of isolates from patients with antibiotic-associated hemorrhagic colitis. *J Clin Microbiol* 2014; **52**: 1607–16.

16 Derde LP, Cooper BS, Goossens H *et al*. Interventions to reduce colonisation and transmission of antimicrobial-resistant bacteria in intensive care units: an interrupted time series study and cluster randomised trial. *Lancet Infect Dis* 2014; **14**: 31–9.

17 Drieux L, Brossier F, Sougakoff W *et al*. Phenotypic detection of extended-spectrum β-lactamase production in Enterobacteriaceae: review and bench guide. *Clin Microbiol Infect* 2008; **14** Suppl 1: 90–103.

18 Lee K, Lim YS, Yong D *et al*. Evaluation of the Hodge test and the imipenem-EDTA double-disk synergy test for differentiating metallo-β-lactamase-producing isolates of *Pseudomonas* spp. and *Acinetobacter* spp. *J Clin Microbiol* 2003; **41**: 4623–9.

19 Doi Y, Potoski BA, Adams-Haduch JM *et al*. Simple disk-based method for detection of *Klebsiella pneumoniae* carbapenemase-type β-lactamase by use of a boronic acid compound. *J Clin Microbiol* 2008; **46**: 4083–6.

20 Glupczynski Y, Huang TD, Bouchahrouf W *et al*. Rapid emergence and spread of OXA-48-producing carbapenem-resistant Enterobacteriaceae isolates in Belgian hospitals. *Int J Antimicrob Agents* 2012; **39**: 168–72.

21 Seifert H, Dolzani L, Bressan R *et al*. Standardization and interlaboratory reproducibility assessment of pulsed-field gel electrophoresis-generated fingerprints of *Acinetobacter baumannii*. *J Clin Microbiol* 2005; **43**: 4328–35.

22 Tenover FC, Arbeit RD, Goering RV *et al*. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995; **33**: 2233–9.

23 Grundmann H, Hori S, Tanner G. Determining confidence intervals when measuring genetic diversity and the discriminatory abilities of typing methods for microorganisms. *J Clin Microbiol* 2001; **39**: 4190–2.

24 Librado P, Rozas J. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 2009; **25**: 1451–2.

25 Empel J, Baraniak A, Literacka E *et al*. Molecular survey of β-lactamases conferring resistance to newer β-lactams in Enterobacteriaceae isolates from Polish hospitals. *Antimicrob Agents Chemother* 2008; **52**: 2449–54.

26 Woodford N, Fagan EJ, Ellington MJ. Multiplex PCR for rapid detection of genes encoding CTX-M extended-spectrum β-lactamases. *J Antimicrob Chemother* 2006; **57**: 154–5.

27 Perez-Perez FJ, Hanson ND. Detection of plasmid-mediated AmpC β-lactamase genes in clinical isolates by using multiplex PCR. *J Clin Microbiol* 2002; **40**: 2153–62.

28 Fiett J, Baraniak A, Mrowka A *et al*. Molecular epidemiology of acquired-metallo-β-lactamase-producing bacteria in Poland. *Antimicrob Agents Chemother* 2006; **50**: 880–6.

29 Woodford N, Turton JF, Livermore DM. Multiresistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. *FEMS Microbiol Rev* 2011; **35**: 736–55.

30 Rogers BA, Sidjabat HE, Paterson DL. *Escherichia coli* O25b-ST131: a pandemic, multiresistant, community-associated strain. *J Antimicrob Chemother* 2011; **66**: 1–14.

31 Breurec S, Guessennd N, Timinouni M *et al*. *Klebsiella pneumoniae* resistant to third-generation cephalosporins in five African and two Vietnamese major towns: multiclonal population structure with two major international clonal groups, CG15 and CG258. *Clin Microbiol Infect* 2013; **19**: 349–55.

32 Deleo FR, Chen L, Porcella SF *et al*. Molecular dissection of the evolution of carbapenem-resistant multilocus sequence type 258 *Klebsiella pneumoniae*. *Proc Natl Acad Sci USA* 2014; **111**: 4988–93.

33 Izdebski R, Baraniak A, Herda M *et al*. MLST reveals potentially high-risk international clones of *Enterobacter cloacae*. *J Antimicrob Chemother* 2015; **70**: 48–56.

34 Granier SA, Leflon-Guibout V, Goldstein FW *et al*. New *Klebsiella oxytoca* β-lactamase genes bla_{OXY-3} and bla_{OXY-4} and a third genetic group of *K. oxytoca* based on bla_{OXY-3}. *Antimicrob Agents Chemother* 2003; **47**: 2922–8.

35 Granier SA, Plaisance L, Leflon-Guibout V *et al*. Recognition of two genetic groups in the *Klebsiella oxytoca* taxon on the basis of chromosomal β-lactamase and housekeeping gene sequences as well as ERIC-1 R PCR typing. *Int J Syst Evol Microbiol* 2003; **53**: 661–8.

36 Papagiannitsis CC, Izdebski R, Baraniak A *et al*. Survey of metallo-β-lactamase-producing Enterobacteriaceae colonizing patients in European ICUs and rehabilitation units, 2008–11. *J Antimicrob Chemother* 2015; **70**: 1981–8.