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Molecular characterization of Danish ESBL/AmpC-producing *Klebsiella pneumoniae* from bloodstream infections, 2018

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ABSTRACT

Objectives: The aim of the study was to molecularly characterize third-generation cephalosporin-resistant *Klebsiella pneumoniae* isolated from bloodstream infections in Denmark in 2018 using whole-genome sequencing (WGS) data, and to compare these isolates to the most common clones detected in 2006 and 2008.

Methods: Sixty-two extended-spectrum beta-lactamase (ESBL)/AmpC-producing *K. pneumoniae* isolates from Danish blood cultures from 2018 were analysed using WGS to obtain multilocus sequence typing (MLST), core genome MLST (cgMLST), resistance profile and phylogeny. These were compared to the most common ESBL *K. pneumoniae* clones detected in 2006 and 2008.

Results: The most common ESBL clone was ST15 CTX-M-15, the DHA-1 enzyme was the most common in AmpC isolates, and the OXA-48-like group was the most common carbapenemase. Thirty-nine different sequence types (STs) were found, with the most frequent being ST14, ST15 and ST37, accounting for 24% of the isolates. The isolates were subdivided into 55 complex types (CTs) of which 49 were singletons, with the most frequent being ST14-CT2080. Two of the CTX-M-15-producing isolates from 2018 belonged to the ST15-CT105/CT3078 clone, which was first detected in 2006.

Conclusions: The ESBL/AmpC *K. pneumoniae* isolates detected in Danish blood cultures belonged to many different types. No dominant clones were circulating in Danish hospitals, but the ST15-CT105/CT3078 CTX-M-15 *K. pneumoniae* clone was seen 13 years after its first detection.

1. Introduction

Klebsiella pneumoniae is known to cause nosocomial infections, such as urinary tract infections, abdominal infections and bacteraemia. It can cause hospital outbreaks, probably because the hospital environment provides multiple niches beneficial for bacterial survival and growth and *K. pneumoniae* is easily transmitted by hands [1]. *K. pneumoniae* has recently been characterized as a complex consisting of six phylogroups, the *K. pneumoniae sensu stricto*, *Klebsiella variicola*, *Klebsiella*

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quasivariicola, Klebsiella quasipneumoniae subsp. *quasipneumoniae*, *Klebsiella quasipneumoniae* subsp. *similipneumoniae* and an unnamed phylogroup [2].

K. pneumoniae is inherently resistant towards aminopenicillins. Furthermore, they can become resistant towards second- and third-generation cephalosporins, most often caused by resistance genes encoding AmpC or extended-spectrum beta-lactamase (ESBL) production placed on plasmids often comprising additional genes causing resistance towards fluoroquinolones and aminoglycosides. In case of combined resistance, infections are more difficult to treat and are associated with increased mortality and morbidity and the ESBL-producing bacteria are therefore placed in Priority 1: critical on the WHO priority pathogens list for Research and Development of new antibiotics [3].

Before 2007, the prevalence of resistance towards second- and third-generation cephalosporins in *K. pneumoniae* from bloodstream infections was less than 5% in average in Denmark, but in 2007 a rise was registered [4]. Detection of a ST15 CTX-M-15-producing *K. pneumoniae* clone started in 2006 in the Capital Region (CR) and led to a regional collection and characterization of the ESBL/AmpC-producing *K. pneumoniae* isolates. In 2008, a national surveillance of ESBL/AmpC-producing *K. pneumoniae* from bloodstream infections was completed and it revealed that the ST15 CTX-M-15-producing clone was widespread in the CR of Denmark [5,6]. Furthermore, spread of an ST16 CTX-M-15-producing *K. pneumoniae* clone was detected in 2008 in the same region [7].

The aim of this study was to molecularly characterize ESBL/AmpC- and carbapenemase-producing *K. pneumoniae* isolates detected in blood cultures in Denmark in 2018 using whole-genome sequencing (WGS) data and to compare the clonal relationship of the findings from 2018 with the most common CTX-M-15 *K. pneumoniae* clones detected in 2006 and 2008, to observe if these clones were still present.

2. Materials and methods

2.1. Demographic data

Denmark is divided into five Regions (NUTs-level 2) — the CR, Region Zealand (RZ), Central Region Denmark (CRD), Region of Southern Denmark (RSD) and Region of Northern Denmark (RND), and the population has for the last 10 years been around 5.5 million people.

2.2. Bacterial isolates

From January 2018 through December 2018, all Departments of Clinical Microbiology (DCM) in Denmark voluntarily submitted third-generation cephalosporin (cefpodoxime, ceftazidime, ceftriaxone or cefotaxime)-resistant *K. pneumoniae* complex member isolates from bloodstream infections to the National Reference Laboratory at Statens Serum Institut (SSI), Denmark. Only one isolate per patient was included in the study.

At SSI, ESBL and/or AmpC phenotypes were identified by a combination disk method using Neo-Sensitabs™ (Rosco, Taastrup, Denmark).

The isolates from 2018 were compared with CTX-M-15-producing *K. pneumoniae* isolates belonging to the two most common clones detected in the collections from 2006 and 2008, two ST15 CTX-M-15-producing *K. pneumoniae* isolates from 2006, six ST15 CTX-M-15-producing *K. pneumoniae* isolates from 2008 and one ST16 CTX-M-15-producing *K. pneumoniae* isolate from 2008 [5–7].

2.3. WGS data analysis

The genomic DNA was extracted (DNeasy Blood and Tissue Kit, Qiagen, Copenhagen, Denmark), with subsequent library construction (Nextera Kit, Illumina, Little Chesterford, UK) and finally by WGS (MiSeq or Nextseq, Illumina) according to the manufacturer's instructions to obtain paired-end reads of 2 × 150 bp in length. Quality control was performed on the raw reads, using the Bifrost pipeline at SSI (https://github.com/ssi-dk/bifrost) with accepted average coverage >30.

The WGS data were either used as raw data or de novo assembled using the assemblies generated using SKESA in Bifrost. The raw reads of all isolates were assembled into draft genomes using SKESA v. 2.2 in the Bifrost pipeline.

For identification of isolates belonging to the species *K. variicola* and *K. quasipneumoniae*, the ribosomal multilocus sequence typing (MLST) scheme (rMLST) based on 53 genes encoding ribosomal proteins was used [8].

Resistance genes were identified using ResFinder version 2.1 [9] (included in the Bifrost pipeline), using a threshold of 100% ID for identifying genes encoding β-lactamases and carbapenemases and 98.00% ID for all other genes encoding transferable antimicrobial resistance.

The draft genome data were also submitted through the batch uploader for the Bacterial Analysis Platform (BAP) to the Center for Genomic Epidemiology (https://cge.cbs.dtu.dk/services/cge/) (CGE, DTU, Kgs. Lyngby, Denmark) [10]. Using the pipeline-version 2.0, the CGE BAP automatically analysed the data with the following tools: Kmerfinder 2.1, ContigAnalyzer 1.0, ResFinder 2.2 and MLST 1.6, resulting in species, ST and resistance gene identification. The MLST (v. 1.6) web tool with the Pasteur MLST scheme was used for identification of STs, as part of the CGE BAP.

To further distinguish the isolates, the SeqSphere+ software (Ridom, Münster, Germany) (using the *K. pneumoniae sensu lato* cgMLST scheme [v. 1.0; 2358 loci]) was utilized to assign complex types (CTs) based on the core genome with a cluster distance threshold of ≤15 allele differences. The cgMLST scheme was able to assign CTs for the species *K. pneumoniae*, *K. variicola* and *K. quasipneumoniae*.

Isolates belonging to new STs were submitted to the Pasteur Institute: https://bigsdb.pasteur.fr/klebsiella/klebsiella.html, and isolates belonging to a new CT were submitted to Seqsphere + for creation of a new CT.

3. Results

3.1. Demographic data for the ESBL/AmpC-producing K. pneumoniae complex isolates

During 2018, 62 ESBL/AmpC-producing *K. pneumoniae* blood culture isolates from 62 patients were collected at SSI, with a regional contribution as follows: the CR (n = 27), RZ (n = 7), RSD (n = 10), CRD (n = 12) and the RND (n = 6) (Table 1).

Gender was distributed with a men/women ratio at 2.88, an average age of 66 years and a median age of 68 years (men: average age 67 years, median 69 years; women: average age 65 years, median 65 years). No ESBL/AmpC-producing *K. pneumoniae* isolates were detected from children (<18 years of age) with bacteraemia.

3.2. Species identification and resistance genes

The 62 isolates were subdivided into 60 *K. pneumoniae* and two *K. quasipneumoniae*.

Of the 62 isolates, five isolates were carbapenemase-producing, 59 isolates were ESBL-producing and five isolates were AmpC-positive.

Table 1
Characterization of ESBL/AmpC-producing *Klebsiella pneumoniae* complex isolates from bloodstream infections 2018, Denmark.

Phenotype	MLST (ST)	cgMLST (CT)	Genotype						No. of isolates	Region (NUTs-2 level)
			Carbapenemases	Extended spectrum beta-lactamases	AmpC beta-lactamases	Fluoroquinolone resistance genes	Aminoglycoside resistance genes	Aminoglycoside/ fluoroquinolone resistance genes		
CPO/ESBL	ST101	CT2089	OXA-48	CTX-M-15		*oqxA, oqxB*	*aac(3)-IIa, aac(6′)-Ib*	*aac(6′)-Ib-cr*	2	CR
	ST147[a]	CT1789	OXA-48	CTX-M-15	DHA-1	*oqxA, oqxB, qnrB4*	*aac(3)-IIa, aph(6)-Id, aph(3″)-Ib*	*aac(6′)-Ib-cr*	1	CR
	ST231	CT2102	OXA-232	CTX-M-15		*oqxA, qnrS1*	*aadA2*	*aac(6′)-Ib-cr*	1	CRD
	ST3457	CT2157	OXA-48	CTX-M-3		*oqxA, oqxB, qnrS1*	*aph(6)-Id*		1	RZ
ESBL	ST14	CT2080		CTX-M-15		*oqxA, oqxB, qnrB1*	*aph(6)-Id, aph(3″)-Ib*		3	CR, CRD
		CT2231		CTX-M-15		*oqxA, oqxB, qnrB1*	*aph(6)-Id, aph(3″)-Ib*		1	RZ
		CT2252		CTX-M-15		*oqxA, oqxB, qnrB1*	*aph(6)-Id, aph(3″)-Ib*	*aac(6′)-Ib-cr*	1	CR
		CT2257		CTX-M-15		*oqxA, oqxB, qnrB1*	*aph(6)-Id, aph(3″)-Ib, aph(3′)-Ia*		1	CR
		CT3077		CTX-M-15		*oqxA, oqxB*	*aph(6)-Id, aph(3″)-Ib*		1	CRD
	ST15[a]	CT105		CTX-M-15		*oqxA, oqxB*	*aph(6)-Id, aph(3″)-Ib*		1	CR
		CT2128		CTX-M-15		*oqxA, oqxB, qnrB1*	*aph(6)-Id, aph(3″)-Ib, aac(3)-IIa*	*aac(6′)-Ib-cr*	1	RND
		CT3076		CTX-M-15		*oqxA*	*aac(3)-IId, aadA2, aph(3′)-Ia*	*aac(6′)-Ib-cr*	1	CR
		CT3078		CTX-M-15		*oqxA, oqxB*	*aph(6)-Id, aph(3″)-Ib*	*aac(6′)-Ib-cr*	1	CR
	ST20	CT2273		CTX-M-154		*oqxA, oqxB*	*aph(6)-Id, aph(3″)-Ib*	*aac(6′)-Ib-cr*	1	RND
	ST25	CT2232		CTX-M-15		*oqxA, oqxB, qnrB1*	*aph(6)-Id, aph(3″)-Ib*		1	RSD
	ST37	CT2710		CTX-M-15		*oqxA, oqxB, qnrB1*	*aph(6)-Id, aph(3″)-Ib, aac(3)-IIb*	*aac(6′)-Ib-cr*	1	CRD
		CT2793		CTX-M-15		*oqxA, oqxB*	*aph(6)-Id, aph(3″)-Ib, aac(3)-IIb*	*aac(6′)-Ib-cr*	1	RSD
		CT3088		CTX-M-14		*oqxA, oqxB, qnrS1*	*aac(3)-IId*		1	RND
		CT3093		CTX-M-14		*oqxA, oqxB*			1	CR
	ST45	CT2195		CTX-M-15		*oqxA, oqxB*	*aph(6)-Id, aph(3″)-Ib, aac(3)-IIa*	*aac(6′)-Ib-cr*	1	CRD
		CT2229		CTX-M-15		*oqxA, oqxB, qnrB1*	*aph(6)-Id, aph(3″)-Ib, aac(3)-IIa*	*aac(6′)-Ib-cr*	1	RSD
	ST46	CT3080		SHV-27		*oqxA, oqxB*	*aadA2*		1	CRD
	ST48	CT829		CTX-M-15		*oqxA, oqxB, qnrB1*	*aph(6)-Id, aph(3″)-Ib*		1	CR
	ST70[a]	CT2127		CTX-M-15		*oqxA, oqxB, qnrB1*[b]	*aac(3)-IId, aac(6′)-Ib*[b], *aph(3″)-Ib*[b], *aph(6)-Ib*[b]	*aac(6′)-Ib-cr*	2	CR, RND
	ST152	CT3082		CTX-M-15		*oqxA, oqxB, qnrB6*	*aac(3)-IIa, aadA16, aph(3′)-Ia, aph(3″)-Ib*	*aac(6′)-Ib-cr*	1	RZ
	ST219	CT3091		CTX-M-15		*oqxA, oqxB, qnrS1*	*aph(6)-Id, aadA2, aph(3′)-Ia, aph(3″)-Ib*		1	CR
	ST231	CT3079		CTX-M-15		*oqxA, oqxB*			1	RSD
	ST268	CT3081		CTX-M-15		*oqxA, oqxB*	*aac(3)-IIa, aph(3′)-Ia, aadA5*	*aac(6′)-Ib-cr*	1	RSD
	ST307	CT932		CTX-M-14		*oqxA, oqxB, qnrB1*		*aac(6′)-Ib-cr*	1	CRD
		CT2110		CTX-M-15	DHA-1	*oqxA, oqxB, qnrB1, qnrB4*	*aac(3)-IIa, aph(6)-Id, aph(3″)-Ib*	*aac(6′)-Ib-cr*	1	CRD
		CT3075		CTX-M-15		*oqxA, oqxB, qnrB1*	*aac(3)-IIa, aph(6)-Id, aph(3″)-Ib*	*aac(6′)-Ib-cr*	1	CR
	ST359	CT2251		CTX-M-15		*oqxA, oqxB, qnrB1*	*aac(3)-IIa, aph(6)-Id*	*aac(6′)-Ib-cr*	1	CR
	ST414[c]	CT3072		CTX-M-15		*oqxA, oqxB*	*aac(3)-IIa, aph(6)-Id, aph(3″)-Ib*	*aac(6′)-Ib-cr*	1	CR
	ST416	CT3089		CTX-M-3		*oqxA, oqxB*			1	CR
	ST474[a]	CT1937		CTX-M-15		*oqxA, oqxB, qnrB1*	*aph(6)-Id, aph(3″)-Ib*		1	RSD
	ST491	CT2276		CTX-M-15		*oqxA, oqxB, qnrB1*	*aac(3)-IIa, aph(6)-Id, aph(3″)-Ib*	*aac(6′)-Ib-cr*	1	RZ
	ST711	CT2198		CTX-M-15		*oqxA, oqxB, qnrB1*			1	RSD
	ST846	CT2256		CTX-M-15		*oqxA, oqxB, qnrB1*		*aac(6′)-Ib-cr*	1	RZ
	ST915	CT3090		CTX-M-15		*oqxA, oqxB*	*aac(3)-IId*		2	CR
	ST985	CT2196		CTX-M-15		*oqxA, oqxB, qnrB1*	*aac(3)-IIa, aph(6)-Id, aph(3″)-Ib*	*aac(6′)-Ib-cr*	2	CRD
	ST999	CT3073		CTX-M-15		*oqxA, oqxB, qnrB1*	*aph(6)-Id, aph(3″)-Ib*		1	CR
	ST1128	CT2193		CTX-M-55		*oqxA, oqxB, qnrS1*	*aph(3′)-IIa*		2	RZ
	ST1564	CT2197		CTX-M-15		*oqxA, oqxB, qnrB1*	*aac(3)-IIa, aph(6)-Id, aph(3″)-Ib*	*aac(6′)-Ib-cr*	1	CR
		CT3086		CTX-M-15		*oqxA, oqxB, qnrB1*	*aph(6)-Id, aph(3″)-Ib*		1	CR
		CT3092		CTX-M-15		*oqxA, oqxB, qnrB1*	*aph(6)-Id, aph(3″)-Ib*		1	CR
	ST1662	CT2954		CTX-M-15		*oqxA, oqxB, qnrS1*	*aph(6)-Id, aph(3″)-Ib, aph(3′)-Ia, aadA2*		1	RSD
	ST2441	CT2126		CTX-M-15		*oqxA, oqxB*	*aac(3)-IId, aadA2*		1	RND
	ST2663[c]	CT2205		SHV-12		*oqxA, oqxB, qnrB1*			1	CRD
	ST2724	CT3071		CTX-M-15		*oqxA, oqxB, qnrB1*	*aac(3)-IIa, aph(6)-Id, aph(3″)-Ib*	*aac(6′)-Ib-cr*	1	CR
	ST3157	CT3087		CTX-M-55		*oqxA, oqxB*			1	CR
	ST3233	CT1936		CTX-M-15		*oqxA, oqxB, qnrB1*	*aac(3)-IId, aph(6)-Id, aph(3″)-Ib*		1	RSD

	ST4015	CT3074	CTX-M-15			*aph(3′)-Ia, aadA2*	1		CR
AmpC	ST873	CT2712		DHA-1	*oqxA, oqxB, qnrB4*		1		RND
	ST1035	CT2709		DHA-1	*oqxA, oqxB, qnrS1*		1		CRD
	ST4017	CT3083		DHA-1	*oqxA, oqxB, qnrB4*		1		RSD
2006	ST15	CT105	CTX-M-15		*oqxA, oqxB*	*aac(3)-IIa, aph(3′)-Ia, aph(3″)-Ib*	*aac(6′)-Ib-cr*	2	CR
2008	ST15	CT105	CTX-M-15		*oqxA, oqxB*			1	CR
		CT105	CTX-M-15		*oqxA, oqxB*	*aac(3)-II, strA, strB*	*aac(6′)-Ib-cr*	4	CR, RZ
		CT105	CTX-M-15		*oqxA, oqxB*	*aac(3)-II, aph(6)-Id, aph(3″)-Ib*	*aac(6′)-Ib-cr*	1	RZ
	ST16	3476	CTX-M-15		*oqxA, oqxB*	*strA, strB, aac(6′)-Im, aac(6′)-Ib*	*aac(6′)-Ib-cr*	1	CR

cgMLTS, core genome multilocus sequence typing; CPO, carbapenemase producing organism; CR, Capital Region; CRD, Central Region Denmark; CT, cluster type; ESBL, extended-spectrum beta-lactamase; MLST, multilocus sequence typing; RND, Region of Northern Denmark; RSD, Region of Southern Denmark; RZ, Region Zealand, ST, sequence type.

[a] ST-repeat 2018 vs. 2006 and 2008.

[b] Only one of the isolates.

[c] *K. quasipneumoniae*.

All five carbapenemase-positive isolates belonged to the OXA-48-group: four OXA-48 and one OXA-232.

The most prevalent ESBL genotype was a $bla_{\text{CTX-M-15}}$ detected in 48 of the 62 isolates, while all the five AmpC-producing isolates harboured the $bla_{\text{DHA-1}}$ gene (Table 1).

Eighty-five percent of the isolates were found containing one or several plasmid-mediated aminoglycoside resistance genes. Furthermore, all isolates, but one, harboured one or several genes encoding fluoroquinolone resistance with the aminoglycoside acetyltransferase variant gene *aac(6′)-Ib-cr* being present in 28 of the 62 isolates (Table 1).

3.3. Phylogenetic analysis

The 62 isolates belonged to 39 STs, with the most frequent being ST14, ST15 and ST37, accounting for 24% of the isolates. Typing by cgMLST revealed 55 different CTs, with 49 singletons and six CTs consisting of more than one isolate. The most common CT with three isolates was CT2080 from the ST14 cluster, while the CTs–CT2089, CT2127, CT2193, CT2196 and CT3090 each consisted of two isolates (Table 1).

3.4. Comparison of the ESBL-producing K. pneumoniae isolates from 2018, 2008 and 2006

The two ST15 CTX-M-15-producing *K. pneumoniae* isolates from 2006 and the six ST15 CTX-M-15-producing *K. pneumoniae* isolates from 2008 belonged to CT105. A single ST15-CT105 CTX-M-15-producing isolate was also detected in 2018. Furthermore, one of the CTX-M-15-producing isolates from 2018 belonged to ST15-CT3078, which was a subtype of ST15-CT105 (11 allele difference). The ST16 CTX-M-15-producing *K. pneumoniae* isolates from 2008 belonged to CT3476. None of the ESBL-producing *K. pneumoniae* isolates from 2018 belonged to ST16 or to CT3476.

4. Discussion

Antimicrobial resistance in *K. pneumoniae* has for several years been of great concern worldwide, because of its ability to obtain plasmids harbouring resistance towards third-generation cephalosporins, aminoglycosides, fluoroquinolones and carbapenems.

From 2006 to 2014, an increase in the prevalence of invasive *K. pneumoniae* isolates resistant to third-generation cephalosporins was observed in all of Europe, but thereafter, no common trend has been registered. Regarding invasive carbapenemase-producing *K. pneumoniae*, a significant increase in prevalence has been observed since 2015 [11].

In our investigation, we found that the most frequent resistance genes in the Danish ESBL *K. pneumoniae* isolates have not changed during the last 11 years and that the $bla_{\text{CTX-M-15}}$ gene remains the most frequent ESBL gene [7]. In our study, four carbapenemase-producing *K. pneumoniae* harboured the OXA-48 enzyme, which is the most frequent enzyme identified in the OXA-48-like carbapenemase group. One carbapenemase-producing *K. pneumoniae* contained the OXA-232 enzyme, which also belongs to the OXA-48-like carbapenemase group and is the third most common OXA-48-like carbapenemase [12].

The *aac(3)-II* gene and the *aac(6′)-Ib-cr* gene were the most prevalent aminoglycoside and fluoroquinolone resistance genes in 2018, which were similar to 2008 [7].

Very few isolates in the 2018 collection belonged to the same STs and further differentiation with cgMLST revealed more than 50 different CTs, with ST14-CT2080 being the most frequent. Isolates belonging to the same CTs were obtained from the same regions in Denmark.

Two OXA-48-producing *K. pneumoniae* isolates belonged to ST101 and one to ST147. Both types have been reported as global high-risk clones and are reported worldwide [12]. Furthermore, one isolate of ST231 OXA-232 was found. This clone was identified for the first time in 2011 in France, and has later been reported from USA and India and as an epidemic clone in South-East Asia [13,14].

The ST15 CTX-M-15-producing *K. pneumoniae* has been isolated from humans in several countries in both Europe and Asia [15]. It has also been described from companion animals and horses [16].

In Denmark, spread of a ST15 CTX-M-15 *K. pneumoniae* clone was first detected in 2006 [5]. In 2008, this clone was detected from patients with bloodstream infections hospitalized in the CR of Denmark [7]. In our study, six ST15 CTX-M-15-producing isolates belonging to the major ST15 clone in 2006 and two ST15 CTX-M-15 isolates from 2008 were typed by cgMLST and were all found to belong to CT105. One ST15-CT105 CTX-M-15-producing isolate and one ST15-CT3078 isolate, which was a subtype of ST15-CT105 (11 allele difference), were detected in the 2018 collection, both from patients hospitalized in the CR. This could indicate a spread of this clone during the 13-year period in the CR. The origin of the ST15-CT105 CTM-X-15 clone was unknown, but from the cgMLST database (www.cgMLST.org) information was available for a ST15-CT105 CTM-X-15 *K. pneumoniae* isolate from 2007. This isolate was found in an American wounded military person (GenBank accession no. GCA_000788005.1).

Generally, Denmark and other Nordic countries have a low prevalence of resistance towards third-generation cephalosporins, carbapenems as well as combined resistance to third-generation cephalosporins, fluoroquinolones and aminoglycosides compared to the rest of Europe [11]. Historically, the proportion of invasive *K. pneumoniae* with phenotypic resistance towards third-generation cephalosporins in Denmark has imitated the increasing trend in the rest of Europe until 2009, followed by a continuous decrease. At the same time, the number of carbapenemase-producing isolates has continuously been low. Since 2014, DANMAP has registered combined resistance towards third-generation cephalosporins, ciprofloxacin and gentamicin in the Danish invasive *K. pneumoniae* isolates, and reported an annual percentage of around 2% [4,17].

The decrease in the third-generation resistance and resistance towards gentamicin in the Danish *K. pneumoniae* isolates may be due to several things, of which the main reason probably is a restrictive use of antibiotics. The relation between selection of ESBL-producing species and the total antibiotic consumption, as well as the specific use of second- and third-generation cephalosporins and fluoroquinolones, led to an implementation of antibiotic stewardship. The stewardship was implemented in 2007 in hospitals with nosocomial ESBL/AmpC *K. pneumoniae* outbreaks [7]. Antibiotic stewardship involved restriction of cephalosporins, fluoroquinolones and carbapenems and a change of empiric treatment from cefuroxime to piperacillin–tazobactam [4,18]. In 2012, The Danish Health and Medicines Authority introduced an antibiotic guideline that included restrictions on the prescription of cephalosporins, fluoroquinolones and carbapenems in primary healthcare and in hospitals. Furthermore, in 2017, the Danish Ministry of Health defined a national action plan and published national goals for the reduced consumption of antimicrobial agents in humans [19,20].

In our study, we only investigated isolates from bloodstream infections, which is a limitation. A more thorough investigation of the clonal dissemination would have been possible if isolates from faecal and urine isolates also had been included.

In conclusion, ESBL/AmpC-producing *K. pneumoniae* bacteraemia isolates from Denmark collected in 2018 belonged to many different types and no clonal dissemination was detected in the blood samples from the Danish hospitals. However, a ST15-CT105/3078 CTX-M-15-producing *K. pneumoniae* clone from 2006 was detected. The numbers of ESBL/AmpC-producing *K. pneumoniae* were at a low and stable level, which may indicate that the different initiatives taken during the 2000s were effective.

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Competing interests

None declared.

Ethical approval

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