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A nationwide genomic study of clinical *Klebsiella pneumoniae* in Norway 2001-2015: Introduction and spread of ESBL facilitated by CG15 and CG307

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Running title:

Genomic study of clinical *Klebsiella pneumoniae* in Norway 2001-2015

Synopsis

Objective

We have used the nationwide Norwegian surveillance program on resistant microbes in humans (NORM) to address longitudinal changes in the population structure *K. pneumoniae* isolates during 2001-15, encompassing the emergence and spread of ESBL-producing *Enterobacterales* (ESBL-E) in Norway.

Material and methods

Among blood (n= 6124) and urinary tract (n=5496) surveillance isolates from 2001-15, we used Illumina technology to whole genome sequence 201 ESBL-producing isolates from blood (n=130) and urine (n=71), and 667 non-ESBL isolates from blood. Complete genomes for four isolates were resolved with Oxford Nanopore sequencing.

Results

In a highly diverse collection*, Klebsiella variicola* ssp*. variicola* caused a quarter of *Klebsiella pneumoniae* species complex bacteraemias. ESBL-production was limited to *K. pneumoniae sensu stricto* (98.5 %). A diverse ESBL population of 57 clonal groups (CGs) were dominated by multidrug resistant CG307 (17%), CG15 (12%), CG70 (6%), CG258 (5%) and CG45 (5%) carrying $bla_{\text{CTX-M-15}}$. Yersiniabactin was significantly more common in ESBL-positive (37.8%) compared to non-ESBL *K. pneumoniae sensu stricto* isolates (12.7%), indicating convergence of virulence and resistance determinants

Moreover, we found a significant lower prevalence of yersinabactin (3.0 %, 37.8 % and 17.3 %), IncFIB (58.7 %, 87.9 % and 79.4 %) and IncFII plasmid replicons (40.5 %, 82.8 % and 54.2%) in *K. variicola* ssp. *variicola* compared to ESBL- and non-ESBL *K. pneumoniae sensu stricto,* respectively.

Conclusion

The increase in Norwegian KpSC ESBLs during 2010-15 was driven by *bla*$_{CTX\text{-}M\text{-}15}$ carrying CG307 and CG15. *K. variicola* ssp. *variicola* was a frequent cause of invasive KpSC infection, but rarely carried ESBL.

Introduction

Klebsiella pneumoniae is an important human pathogen [1] and acknowledged as a key host for the spread of antimicrobial resistance (AMR)[2, 3]. The global spread of multidrug resistance (MDR) [4] *K. pneumoniae* is closely linked to the spread of extended-spectrum β-lactamases (ESBLs) and carbapenemases. This has been facilitated by successful clonal lineages or clonal groups (CGs) such as CG258, CG15 and CG307 [5-7], and horizontal gene transfer (HGT) [2, 8], fueled by antibiotic selection [9].

Whilst *K. pneumoniae* typically causes severe infections in vulnerable hospitalized patients [1], some hypervirulent (HV) clones cause community acquired invasive infections, often in healthy individuals [10]. HV-clones cluster in CG23, CG65 and CG86, and harbour capsular loci K1 or K2, siderophores and other virulence factors supporting colonization, tissue invasion and immune evasion [10, 11]. High-risk *K. pneumoniae* clones, categorized as either MDR or HV, rarely display both traits [12]. However, in recent years, convergence of the two traits has been reported [13].

K. pneumoniae is a highly diverse species, and the term *K. pneumoniae* species complex (KpSC) has been introduced to encompass seven closely related taxa [14], of which *K. pneumoniae sensu stricto*, *Klebsiella variicola* and *Klebsiella quasipneumoniae* are the most frequently reported in human clinical samples [15, 16].

Most molecular epidemiological studies of KpSC have focused on outbreaks or isolates with particular characteristics such as AMR or virulence, most often with a cross-sectional study design. Thus, there is a need for longitudinal studies, including both resistant and susceptible isolates, to improve our understanding of the population dynamics in clinical KpSC isolates.

Here, we have used the Norwegian surveillance program on resistant microbes (NORM) during 2001-2015 to address the longitudinal dynamics of KpSC clinical isolates, dominant CGs and their associations with clinically important AMR- and virulence determinants.

The nationwide data show that the emergence of ESBL-producing KpSC clinical isolates in Norway has been dominated by MDR $bla_{CTX\text{-}M\text{-}15}$ carrying *K. pneumoniae sensu stricto*, with CG15 and CG307 as major lineages. *K. variicola ssp. variicola* is a significant contributor to KpSC bacteraemias, but rarely carries ESBL genes in this geographical setting.

Material and methods

Bacterial isolates

NORM monitors AMR in *Klebsiella* spp. isolated from blood and urine isolates. Antimicrobial susceptibility is performed and interpreted according to EUCAST guidelines and breakpoints [17]. Isolates with reduced susceptibility to cefotaxime and/or ceftazidime are categorized as ESBL or non-ESBL based on phenotypical ESBL-testing [18]. Isolates are stored locally at -80°C at the participating laboratories [18].

All putative ESBL-producing KpSC blood (n=149) and urine isolates (n=91) from 2001-2015 registered in the NORM database were included in the study. For comparison, a subset of non-ESBL blood culture isolates (n=815) were included. To achieve a balanced sample for each year and to maintain a representative geographical and temporal distribution, consecutive entries were selected from each

laboratory according to the following key: 2001, all isolates; 2005, every 2 out of 3; 2009, every 1 out of 2; and 2015, every 1 out of 3 (Figure S1).

Isolates registered as either *K. pneumoniae* or *Klebsiella* spp. were included. Species identification was confirmed by MALDI-TOF MS (MBT Compass Library DB-6903, Bruker Daltonik), and subsequently by whole genome sequencing. Only KpSC isolates were included for further analyses. Antimicrobial susceptibility profiles, laboratory, year and source of isolation were retrieved from the NORM database. For isolates with discordant ESBL geno- and phenotype, the phenotype was confirmed using the combined disc method (Becton Dickinson, New Jersey, USA) [19]. Colistin MIC was determined by broth microdilution using Sensititre FRCOL plates (Thermo Fisher Scientific, East Grinstead, UK) according to manufacturer's instructions.

Whole genome sequencing and *in silico* analysis

See supplementary methods for details. Paired-end reads (300 or 125 bp) were generated for all isolates using Illumina MiSeq and Illumina HiSeq platforms, respectively. Selected isolates were also long-read sequenced on a MinION Mk1B device (Oxford Nanopore Technologies). Unicycler v0.4.8 [20] was used for all assembly.

Kleborate v2.0.4 [21] was used to identify species, sequence type (ST), virulence loci and AMR genes (CARD database v3.0.8 [22]). Kaptive [23] was used to identify capsule (K) biosynthesis loci reporting calls with confidence level "Good" or higher.

Read-sets from putative ESBLs with no definite ESBL gene were investigated using SRST2 v.2.0.4 [24] with CARD database v3.0.8, as reads containing ESBL sequences may have been discarded during

assembly, and/or assembled across multiple contigs. Plasmid replicons were identified with SRST2 v0.2.0 using the PlasmidFinder database version 2021-01-13 [25].

A core chromosomal single-nucleotide variant (SNV) alignment of all genomes was generated by mapping short-reads to the chromosome of the ST23 reference genome NTUH-K2044 (Genbank accession NC_01273.1) using RedDog V1beta.11 [26]. A maximum likelihood (ML) phylogeny was inferred from the resulting alignment using FastTree v2.1.10 [27].

CGs were defined from the phylogeny using patristic distance cut-off of 0.04 [13] and named according to the dominant ST within each CG. However, CGs dominated by ST14 and ST340 were named CG15 and CG258, as these are more commonly known [2, 28]. An alignment of all CG307 genomes was generated with RedDog V1beta.11, using the closed ST307 genome (Genbank accession CP073627) as the mapping reference, and subsequently passed to RAxML v8.2.10 [29] to infer a CG307 clone-specific ML phylogeny.

Data availability

The 868 KpSC short-read and three long-read sequence files have been deposited in the European Nucleotide Archive under BioProject PRJEB27256 (Table S1). The four hybrid-assembled completed genomes have been deposited in GenBank (Table S1) under accession numbers CP073791-CP073796, CP073627-CP073629, CP073783-CP073787 and CP073788-CP073790.

Definitions

MDR was defined as phenotypic resistance to agents in three antimicrobial classes [4]. HV was defined as either a) the presence of *rmpA* or *rmpA2*; and/or b) the presence of aerobactin (*iuc*) and salmochelin (*iro*) [30]. ESBL isolates were defined as either having known ESBL genes (i.e. $bla_{\text{CTX-M}}$, $bla_{\text{SHV-2}}$, $bla_{\text{SHV-5}}$, $bla_{\text{SHV-12}}$, $bla_{\text{SHV-18}}$ and $bla_{\text{SHV-24}}$), or in absence of known ESBL genes, a confirmed ESBL

phenotype. Isolates with plasmid-mediated AmpC genes only or carbapenemase-encoding genes (regardless of ESBL gene presence) were excluded from further analysis.

Data handling and statistical analysis

Data analysis and statistics was done using R version 4.0.2 (2020-06-22) [31]. Distribution differences were calculated with Fisher exact test, with Benjamini-Hochberg correction for multiple testing when necessary. $p < 0.05$ was considered statistically significant.

Ethics

The study was approved by the Regional Committee for Medical and Health Research Ethics (Reference: 2017/1185-3).

Results

We received 954/1,055 (90.4%) of requested isolates of which 223 putative ESBL-producing isolates (blood, n=144; urine, n=79) and 667 non-ESBL blood isolates were confirmed as KpSC by MALDI-TOF MS and WGS (Figure S1). Known ESBL genes were detected in 192/223 (86%) putative ESBL isolates. ESBL phenotype was confirmed in nine additional isolates, resulting in an ESBL group consisting of 201 isolates (blood, n=130; urine, n=71). Six isolates with carbapenemase genes and one isolate with a plasmid-mediated AmpC gene only were excluded (Figure S2). The dataset can be explored at https://microreact.org/project/4dBcaZsZmKoAzvatzPaGds.

Phylogenetic diversity in ESBL and non-ESBL KpSC populations

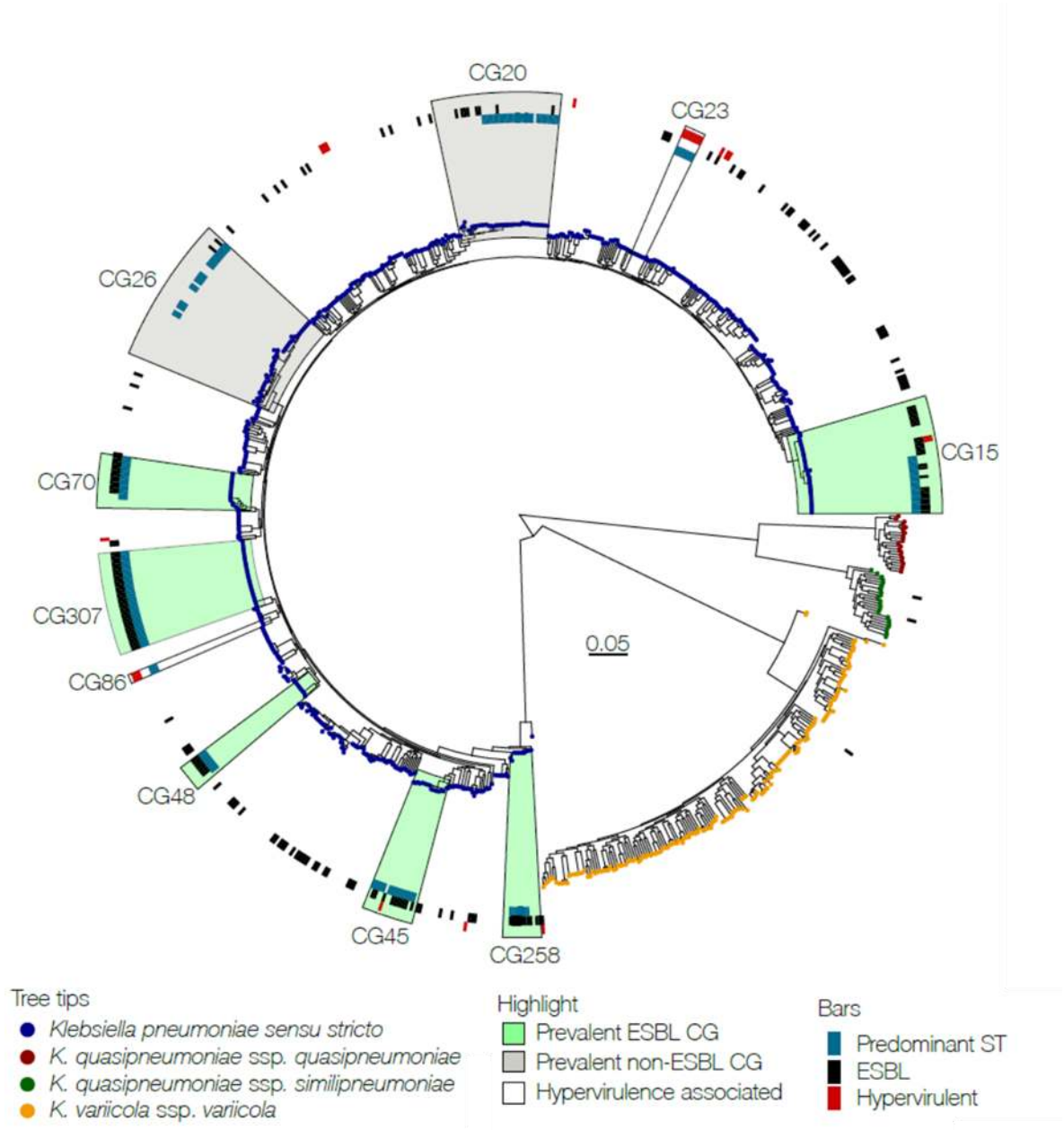
The species distributions in the ESBL and non-ESBL groups were different ($p<0.0001$) (Table 1). The ESBL group consisted of 98.5% *K. pneumoniae sensu stricto*. In contrast, the non-ESBL group isolates comprised *K. pneumoniae sensu stricto* (69.1%), *K. variicola* ssp. *variicola* (24.5%), and *K. quasipneumoniae* ssp. *similipneumoniae* (3.3%) and *K. quasipneumoniae* ssp. *quasipneumoniae* (3.1%). *K. variicola* ssp. *tropica, K. africana* or *K. quasivariicola* were not detected (Table 1, Figure 1).

Table 1. ESBL and non-ESBL groups: species distribution, clonal groups (CGs), and sequence types (STs) numbers

Species identification	ESBL isolates (%)	ESBL (CGs /STs)	non-ESBL isolates (%)	non-ESBL (CGs/STs)	p[1]
K. pneumoniae sensu stricto	198 (98.5)	54/70	461 (69.1)	136/222	
K. variicola ssp. *variicola*	1 (0.5)	1/1	163 (24.5)	80/115	
K. quasipneumoniae ssp. *similipneumoniae*	2 (1)	2/2	22 (3.3)	20/20	
K. quasipneumoniae ssp. *quasipneumoniae*	-	-	21 (3.1)	10/20	
K. pneumoniae species complex	201	57/73	667	246/377	<0.0001

[1]**Fisher exact test for difference in species distributions between groups**

The 868 KpSC isolates were phylogenetically highly diverse with a total of 413 different STs assigned to 261 CGs (Table S1). The Simpsons diversity indices for STs were 0.95 for the ESBL group and 0.99 for the non-ESBL group, respectively.



195

Figure 1. Maximum likelihood tree of the 868 *K. pneumoniae* species complex genomes. Tips are colored by *Klebsiella* species. CGs each representing >5% of isolates either in the ESBL or the non-ESBL group, are highlighted in green and grey, respectively. CGs commonly associated with hypervirulence are highlighted without color. The highlighted areas include all genomes on the most recent common ancestor node of the CG indicated. The circles from inner to outer show genomes with the predominant sequence type (ST, blue bars) within the CG, presence of ESBL-encoding genes (black bars) and isolates meeting the study definition of hypervirulence (red bars).

196 The ESBL group (n=201) consisted of 73 STs and 57 CGs (mean number of isolates per CG 3.53, range
197 1-34). CG307 was the most prevalent (16.9%, n=34; ST307) followed by CG15 (12.4%, n=15; ST14,
198 ST15 and ST627), CG70 (6.5%, n=13; ST70), CG258 (5.0%, n=10; ST11, ST340 and ST437) and CG45
199 (5.0%, n=10; ST45 and ST2954). The remaining CGs represented less than 5% of isolates each (Figure
200 1).
201
202 Among the 667 non-ESBL blood culture isolates there were 377 STs and 246 CGs (mean number of
203 isolates per CG 2.71, range 1-42). CG26 (6.3%, n=42; 11 STs) and CG20 (5.2%, n=35; 6 STs) were the
204 most prevalent. In the remaining 244 CGs (88.5%, n=590) each CG represented less than 5% of the
205 isolates. Ten (1.5%) HV-associated isolates, CG23 (n=7) and CG86 (n=3), were observed. CG307 was
206 absent in the non-ESBL group, while the other major ESBL CGs were present in low numbers; CG15
207 (2.1%, n=14), CG70 (0.7%, n=7), CG45 (1%, n=7) and CG258 (0.4%, n=3). In total, 33 of 57 CGs (57.9
208 %) in the ESBL group were present in the non-ESBL group.
209

210 ## *Temporal trends*

211 The globally successful ESBL CG15 was first observed in this study in 2003, in a urine specimen,
212 becoming the most prevalent CG in blood culture samples between 2009 and 2012. From 2012, the
213 increase in ESBLs was associated with the emergence of CG307. Urine isolates exhibited greater
214 diversity of CGs compared to blood, but all prevalent urine CGs were also represented in blood
215 cultures isolates, albeit several in low numbers (Figure 2). There were no apparent CG-trends in the
216 non-ESBL group.

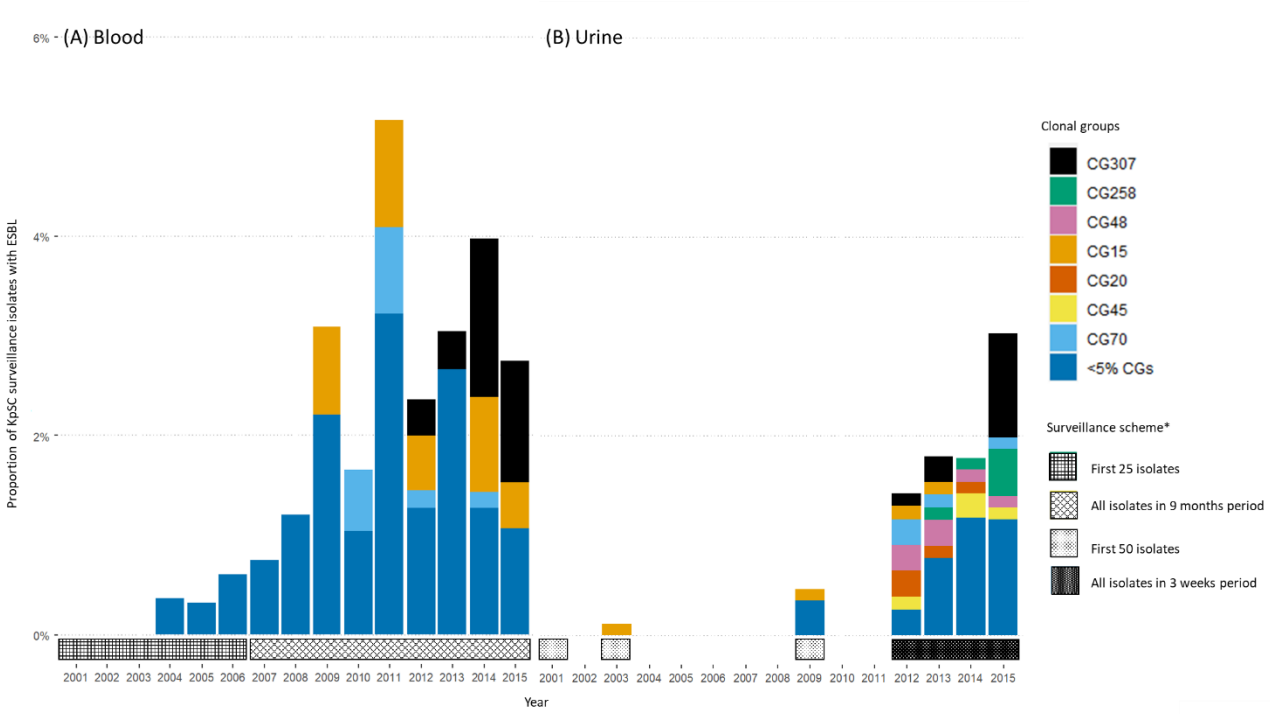


Figure 2. Temporal distribution of *Klebsiella pneumoniae* species complex (KpSC) ESBL clonal groups (CGs) in blood (A) and urine (B) as proportion of the surveillance for each year. Distribution of CGs in blood and urine separated by most prevalent CGs and other CGs. *The surveillance starts in January each year.

CG307 was first observed in 2012 in two of the six NORM surveillance regions, and present in five regions by 2014 (Figure 3A), representing 44.4% of blood- and 34.6% of urine ESBL-isolates in 2015. CG70, CG258 and CG45 emerged in the same period, but did not expand to the same degree. Chromosomal single-nucleotide variants (SNVs), temporal and geographical distribution of the most prevalent CGs are shown in Table S5. A core genome phylogenetic analysis of CG307 indicated independent occurrences of isolates in 2012-2013, while isolates from 2014-2015 seem to represent two clonal expansions (red and blue boxes figure 3B). This is supported by the Bayesian phylodynamic analysis of global CG307 (including 30 of the genomes reported here), adapted from Wyres *et al*. [6], showing that the most recent common ancestor for the two proposed clonal expansions date back to 2009 (Figure S3).

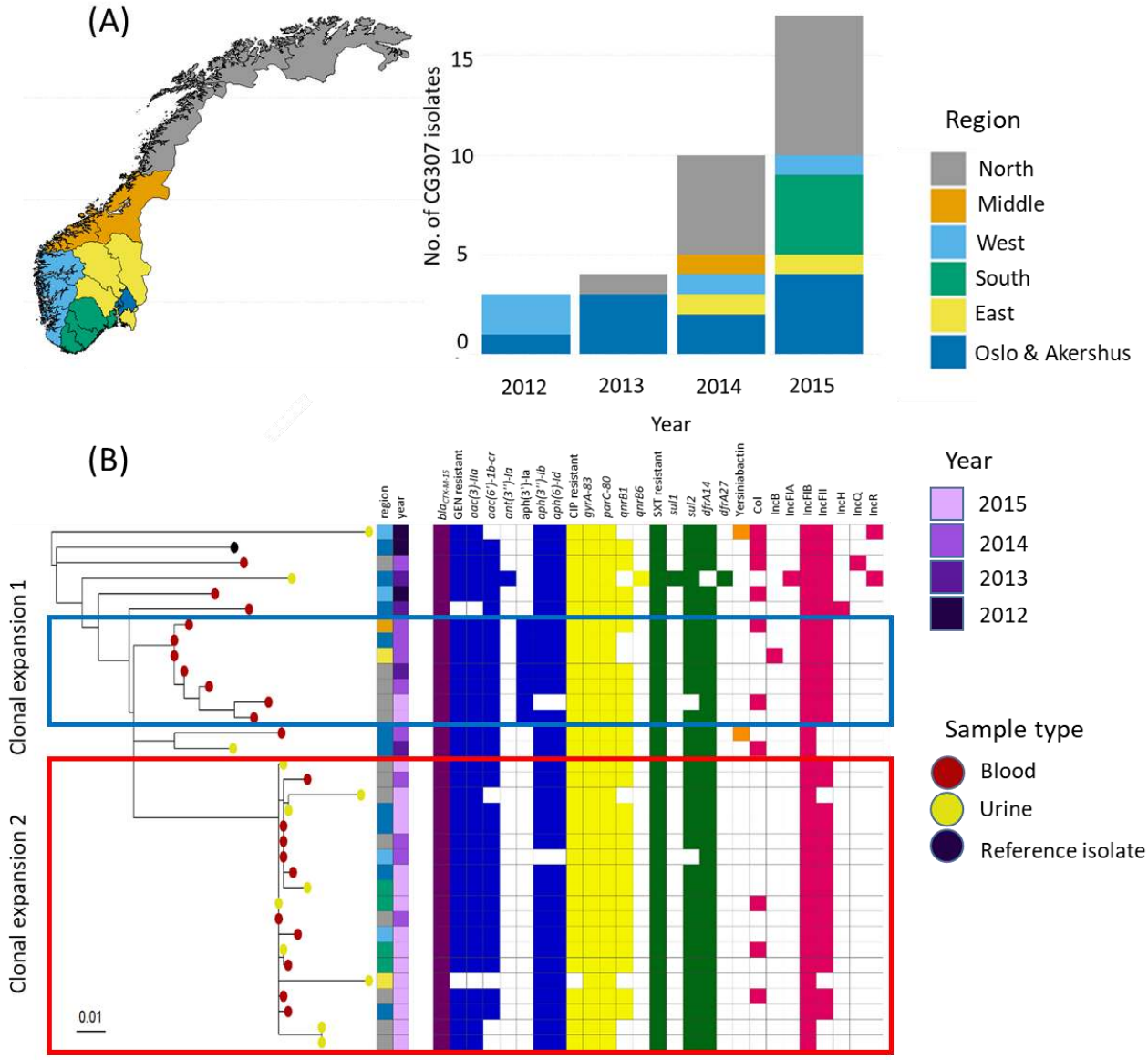


Figure 3 Epidemiology and phylogeny of CG307. (A) Map of Norway with surveillance regions as defined by the Norwegian surveillance program on resistant microbes (NORM), with bar plot showing number of CG307 isolates per region per year. **(B)** CG307 core genome phylogeny with metadata, distribution of AMR determinants, virulence determinants and replicon families. Red and blue boxes mark proposed clonal expansions.

Antimicrobial resistance -– phenotype and genotype

All ESBL group isolates had an ESBL phenotype. Overall, *bla*$_{\text{CTX-M-15}}$ was the most prevalent genotype (n=146; 72.6%), more dominant in high prevalence CGs (93.5%) compared to other CGs (55%, p <0.001). The remaining ESBL-genotypes comprised a diversity of *bla*$_{\text{CTX-M}}$ (n=22; 10.9 %) or *bla*$_{\text{SHV}}$

(n=22; 10.9 %) (Figure 4). Reduced susceptibility to meropenem were found in two ESBL isolates (CG258) and one non-ESBL isolate (CG515), all without carbapenemase genes.

In the ESBL group, reduced susceptibility to gentamicin, ciprofloxacin and trimethoprim-sulfamethoxazole was found in 54.7% (n=110), 72.1% (n= 145) and 88.1% (n=177) of the isolates, with at least one corresponding AMR determinant found in 95.5 % (n =105), 97.8% (n=142) and 96.6 % (n= 171) of the isolates, respectively.

Prevalent AMR-determinants are shown in Figure 4. Of note, *armA* (n=1) and *rmtG* (n=1) encoding 16S-rRNA methylases were rare, while *aac(6')-Ib-cr* which may reduce susceptibility to both aminoglycosides and quinolones was found in 48.3 % (n= 97) of the ESBL isolates. Interestingly, *aac(6')-Ib-cr* was frequently found along with other determinants, in particular in 82.5 % (n=66) and 84.0 % (n=68) of isolates carrying *aac(3)-IIa* and *qnrB1*, respectively.

Colistin resistance determinants were rare. Three ESBL isolates carried *mcr-9.1* (n=2) or a truncated chromosomal gene *mgrB* (n=1) where only the isolate with truncated *mgrB* showed an elevated MIC of 16 mg/L for colistin.

In the non-ESBL group, reduced susceptibility to gentamicin, ciprofloxacin and trimethoprim-sulfamethoxazole was observed in 0.9% (n=5), 2.5% (n=17) and 9.4% (n=63) of the isolates, with at least one corresponding AMR determinant found in 80.0 % (n =4), 58.8% (n=10) and 60.3 % (n= 38), respectively. Prevalent AMR-determinants are shown in Figure 4.

MDR was frequent in the ESBL group, (70.1 %, n= 141) compared to the non-ESBL group (0.3 %, n=2), ($p< 0.001$). Notably, 83.0 % (n=117) of MDR ESBL isolates were carrying $bla_{CTX\text{-}M\text{-}15}$. Only 11.2% (n=18) of ESBL isolates were susceptible to all three of gentamicin, ciprofloxacin and trimethoprim-sulfamethoxazole.

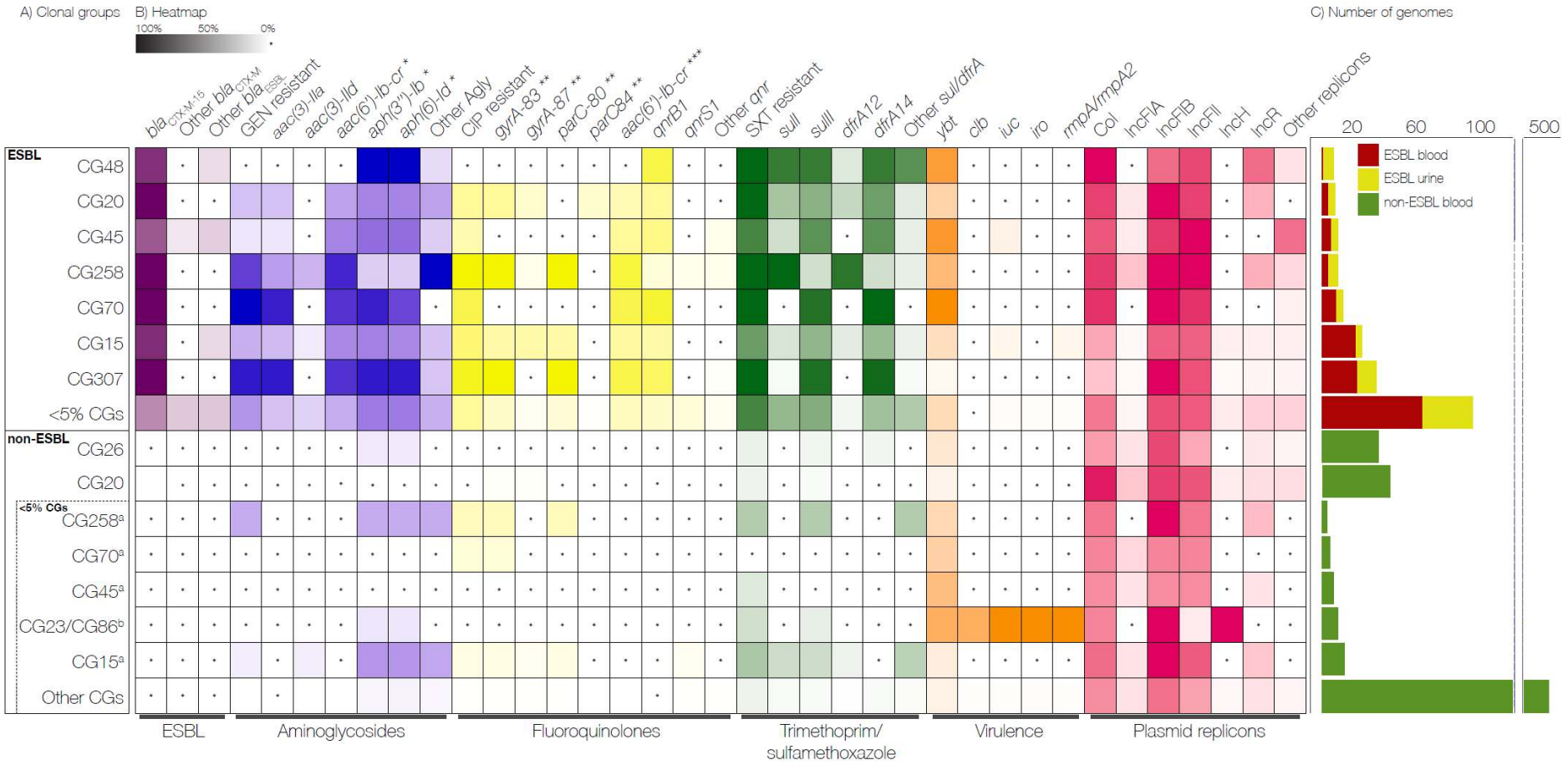


Figure 4. Distribution of resistance determinants, virulence factors and plasmid replicons among clonal groups (CGs). A) Distribution of CGs in the ESBL and non-ESBL groups, separated by most prevalent CGs (each representing >=5% of isolates in group) and other CGs. In the non-ESBL group, <5% CGs which were the **a)** most prevalent CGs from the ESBL group and the **b)** hypervirulence-associated CG23 and CG86 are shown separately. **B)** The intensity of the box shading indicates the percentage of genomes harboured determinant. White shading with a black dot indicates that there are no determinants present. For each antibiotic class, the presence of resistant phenotype and resistance determinants are indicated. **GEN** = gentamicin, **CIP** = ciprofloxacin, **SXT** = trimethoprim-sulfamethoxazole, **AgIy** = acquired aminoglycoside resistance genes, ***** Does not confer resistance to gentamicin, ****** Chromosomal mutation position, ******* May reduce susceptibility to both aminoglycosides and fluoroquinolones, **C)** Total number of genomes in the ESBL and non-ESBL groups.

Diversity in capsule loci and virulence determinants

Capsule loci (KLs) were identified in 73.6 % (n=148, 38 KLs) of the ESBL isolates and 54.3 % (n=362, 87 KLs) of the non-ESBL isolates. KL102 (13.4 %, n=27) was the most prevalent KL among the ESBL isolates, mainly associated with CG307 (n=25). The HV-associated KL1 and KL2 were rare, detected in 5.5 % of ESBL (KL2, n=11) and 3.7% of non-ESBL (KL1, n=11; KL2, n=14) isolates, respectively (Table S2).

Eighteen isolates (2.1 %), all *K. pneumoniae sensu stricto,* met the HV definition, where seven of 15 non-ESBL HV isolates belonged to CG23 (Table S3). Long-read sequencing of one CG133 (ST420) (Genbank accession CP073783-CP073787) ESBL isolate showed $bla_{\text{CTX-M-15}}$ to be situated on an IncFII plasmid without any of the virulence loci. However, two ST15 ESBL isolates (BioSample accession SAMEA5063299, SAMEA5063230), harboured the iuc and rmpA2 virulence loci, as well as blaCTX-M-15 on the same mosaic plasmid, as described by Lam et al. [32].

The distribution of virulence determinants is shown in Figure 4 and Table S3. Yersiniabactin was the most prevalent acquired siderophore in *K. pneumoniae sensu stricto* isolates, and more dominant in ESBL isolates (37.8%, n=75) compared to non-ESBL isolates (17.3 %, n=80) ($p < 0.001$). Notably, among *K. pneumoniae sensu stricto* non-ESBL isolates, there was higher prevalence of yersiniabactin in CGs that were also found in the ESBL group compared to other CGs (29.4 % and 11.0% respectively, $p<0.0009$). Only 5/163 (3.1 %) non-ESBL *K. variicola spp. variicola* had yersiniabactin. While no ESBL isolates had the genotoxin colibactin, it was present in 11 non-ESBL isolates (CG23, n=7; CG133, n=2; CG417; CG643).

Plasmid replicon patterns in ESBL and non-ESBL KpSC populations

Fifteen plasmid replicon families were identified in the ESBL group. IncFIB (87.9%, n=176) and IncFII (82.8%, n= 164) were the most common. In the non-ESBL group, fourteen plasmid replicon families dominated by IncFIB (72.0%, n=480), IncFII (48.7%, n=325), IncFIA (15.0%, n= 100), and IncR (14.5%, n= 97) were identified. Twelve plasmid replicon families were found in both groups (Table S4).

Interestingly, IncFIB and IncFII were more abundant in *K. pneumoniae sensu stricto* in the ESBL group compared to the non-ESBL group ($p<0.001$ and $p<0.001$, respectively). Additionally, in the non-ESBL group IncFIB and IncFII were significantly more common in *K. pneumoniae sensu stricto* compared to the other species ($p<0.001$ and $p<0.03$, respectively, Table 2).

Table 2. Distribution of prevalent replicon types in *Klebsiella pneumoniae sensu stricto* and *K. variicola* ssp. *variicola*

	ESBL	Non-ESBL		p-value*	
Replicon type	**A.** *K. pneumoniae sensu stricto* (n=198)	**B.** *K. pneumoniae sensu stricto* (n=461)	**C.** *K.variicola* ssp. *variicola* (n=163)	A vs B	B vs C
Col	63.1% (125)	63.3% (292)	47.9% (78)	NS	0.006
IncFIA	6.1% (12)	14.3% (66)	17.8% (29)	0.004	NS
IncFIB	87.9% (174)	79.4% (366)	58.3% (95)	0.001-	0.001
IncFII	82.8% (164)	54.2% (250)	40.5% (66)	0.001	0.03
IncR	18.2% (36)	14.1% (65)	12.3% (20)	NS	NS

*Fisher exact test with Benjamini & Hochberg correction for multiple testing. NS – not significant.

Discussion

We have used the nationwide Norwegian AMR surveillance framework to perform a population structure analysis of all ESBL producing KpSC blood and urine isolates as well as a representative collection of non-EBSL blood isolates during 2001-15 for comparison. The combined use of WGS and

317 national registry data, allowed the analysis of temporal and geographical trends in the species
318 distribution, phylogeny, AMR and virulence determinant content in KpSC clinical isolates during a
319 period when ESBL-producing *Enterobacterales* gained foothold in Norway.

320

321 Firstly, we noted a significant difference in species distribution between the ESBL and non-ESBL
322 group. While the ESBL-group was essentially dominated by *K. pneumoniae sensu stricto*, *K. variicola*
323 ssp. *variicola* accounted for 24.5% of the non-ESBL group. This is in line with findings in ESBL-
324 producing or MDR KpSC-strain collections from the USA [33] and the British Isles [33, 34], dominated by *K.*
325 *pneumoniae sensu stricto*. Two studies (Sweden 2007-09, single centre [16] and Japan 2014-17, two-
326 center) of consecutive blood KpSC isolates, both with low prevalence of ESBL, showed similar species
327 distributions compared to our results.

328

329 As gastrointestinal colonization is considered the primary source of the majority of KpSC bloodstream
330 infections [35, 36], we expect that the observed proportion of *K. variicola* ssp. *variicola* among blood
331 isolates reflects the ratio of gut colonization in the patient population. This is supported by a recent
332 Norwegian study where 16.3% of 2,975 healthy adults had KpSC in faecal screening samples, of which
333 28% were *K. variicola spp. variicola* [37]. While *K. pneumoniae sensu stricto* is the predominant KpSC
334 species reported in other gut carriage studies, carriage rates in the range of 10-20% of *K. variicola*
335 have been shown for intensive care patients [35] and pregnant women in low-income countries [30]. As a
336 frequent gut resident, one could expect *K. variicola spp. variicola* to acquire ESBL-encoding plasmids
337 and genes *in vivo*. To our knowledge there are no experimental studies supporting any mechanisms
338 explaining the low abundance of ESBL in *K. variicola spp. variicola* compared to *K. pneumoniae sensu*
339 *stricto*.

340

ESBL rates increased in clinical KpSC isolates during the study period, from 0% in 2001 to 3.1% in 2015 [18]. Our temporal data show an increasing predominance of $bla_{CTX\text{-}M\text{-}15}$ accompanied by an increasing co-resistance to other clinical important antibiotics. An overall increase in ESBL and MDR rates in clinical isolates of KpSC was observed in other European countries in the same period [38]. AMR determinants, as well as phenotypic resistance against gentamicin, ciprofloxacin and trimethoprim-sulfamethoxazole, were rare among non-ESBL isolates in our study. This observation strongly suggest that the overall increase in Norwegian MDR KpSC is driven by the expansion of ESBL-producing *K. pneumoniae sensu stricto*, in particular $bla_{CTX\text{-}M\text{-}15}$ carrying CGs, such as CG15 and CG307.

Our KpSC strain collection is characterized by a large clonal diversity, both in the ESBL and non-ESBL groups, throughout the study period. The most striking shift in the temporal data, in addition to increasing ESBL rates, is the introduction and subsequent spread of CG307 since 2012. The CG307 phylogenetic analyses based on our dataset reveal nationwide expansion of this clone, which seems to be closely related to international isolates as previously shown by Wyres *et al*. [6]. Notably, we have not observed non-ESBL CG307. In contrast, clinical isolates of CG15, a frequent carrier of ESBL, were also present without ESBL.

The observed clonal diversity within the ESBL group and the increasing abundance of CG307 and CG15, is in line with results reported by Moradigaravand *et al.* in the British isles [34] and Long *et al.* in the USA [33] during 2001-2011 and 2011-2015 , respectively. In contrast to their findings, CG258 was less frequently detected in our study. CG307 is still playing an important role in the dissemination of ESBL KpSC in Norway, as confirmed by WGS of blood culture ESBL isolates reported to NORM in 2019, where CG307 (29.2%) remains the dominant CG [18]. While the prevalence of carbapenemase-producing KpSC in Norway is still low [18], the establishment of ESBL CG307 is a cause for concern, as this clone has shown to be a well prepared host for carbapenemase genes in other settings [7].

366

367 Our results suggest that HV KpSC, including CG23 and CG86, are rare in clinical isolates in Norway.
368 This is also in line with the recent population structure analysis of Norwegian KpSC faecal carrier
369 isolates [37]. Yersiniabactin, usually transferred by integrative conjugative elements (ICEs) [39], is the
370 most prevalent virulence-associated gene, mainly found in *K. pneumoniae sensu stricto* isolates.
371 Notably, we observed a significantly higher prevalence of yersiniabactin in ESBL (37.8%) compared to
372 non-ESBL (17.3 %) *K. pneumoniae sensu stricto* isolates, in contrast to previously published results [39].
373 Other virulence determinants were uncommon and played no dominant role in the examined KpSC
374 population. However, as previously reported by Lam *et al*., virulence-encoding genes were found in
375 convergence with $bla_{\text{CTX-M-15}}$ on mosaic plasmids in two ST15 isolates [32].

376 Our data demonstrate that the replicon families IncFIB and IncFII, frequently associated with ESBL
377 genes [40], were common both in ESBL (87.9% and 82.8 %) and non-ESBL (79.7% and 54.2 %) *K.*
378 *pneumoniae sensu stricto* isolates. These replicons were also present in *K. variicola* ssp. *variicola*
379 (58.3% and 40.5 %), but significantly less prevalent compared to non-ESBL *K. pneumoniae sensu*
380 *stricto*.

381

382 Importantly, our data support the notion that there seems to be a higher propensity for certain *K.*
383 *pneumoniae sensu stricto* clonal groups to acquire mobile genetic elements, represented by ESBL-
384 encoding plasmids and yersinabactin-linked ICEs, compared to *K. variicola* ssp. *variicola*. This is
385 concordant with Wyres et al. [13] showing some KpSC clones to be generally better at acquiring genetic
386 material via horizontal gene transfer than others. The observations need further investigations
387 including experimental studies of underlying mechanisms.

388

389 The strength of this study lies in the use of the comprehensive unselected national surveillance data
390 collected over a 15-year period, encompassing the introduction of ESBL, and by using WGS gaining
391 detailed insight into genomic epidemiological features. As we opted for temporal and geographical
392 diversity, we have not done a randomized selection of non-ESBL isolates, which may have introduced
393 a bias in estimating the prevalence of significant CGs or genetic determinants in recent years. The
394 lack of urine ESBL isolates in the periods 2004-2008 and 2010-2011 may also conceal the early
395 appearance of significant CGs.

396

397 In conclusion, the increase of ESBL and clinically relevant co-resistance in *K. pneumoniae sensu stricto*
398 in Norway during the study period is closely linked to $bla_{\text{CTX-M-15}}$ carrying CGs, where CG307 and CG15
399 have played key roles. Yersinabactin and ESBL-encoding mobile genetic elements are uncommon in
400 clinical isolates of *K. variicola* ssp. *variicola* compared to *K. pneumoniae sensu stricto*. Susceptible *K.*
401 *variicola* ssp. *variicola,* however, is a significant pathogen causing one out of four cases of KpSC
402 bacteraemia in Norway.

403

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407

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411

412 **Transparency:**

413 None to declare

414

415 **Contributions:**

416 Study conceivement: A.F, Ø.S, A.S, G.S.S, I.H.L; whole genome sequencing: R.B and E.B.; data analysis:
417 A.F and M.A.K.H; manuscript: A.F and M.A.K.H. All authors contributed to data interpretation, read
418 and commented on the manuscript. Collaborators provided isolates and commented on the final
419 manuscript.

420

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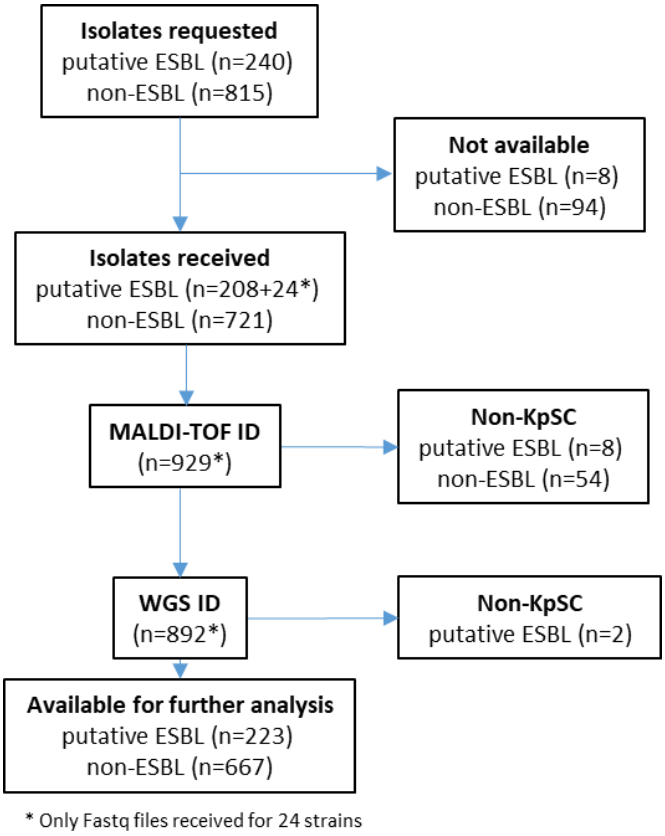
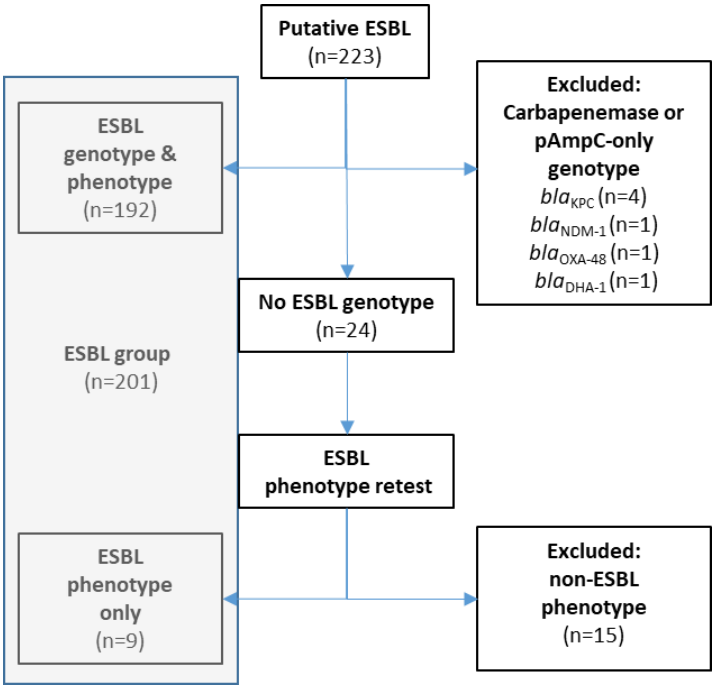
Supplementary figure S1 – Inclusion of isolates.



Supplementary figure S2 – Verification of putative ESBLs



Supplementary figure S3 – Bayesian phylogeny of global CG307

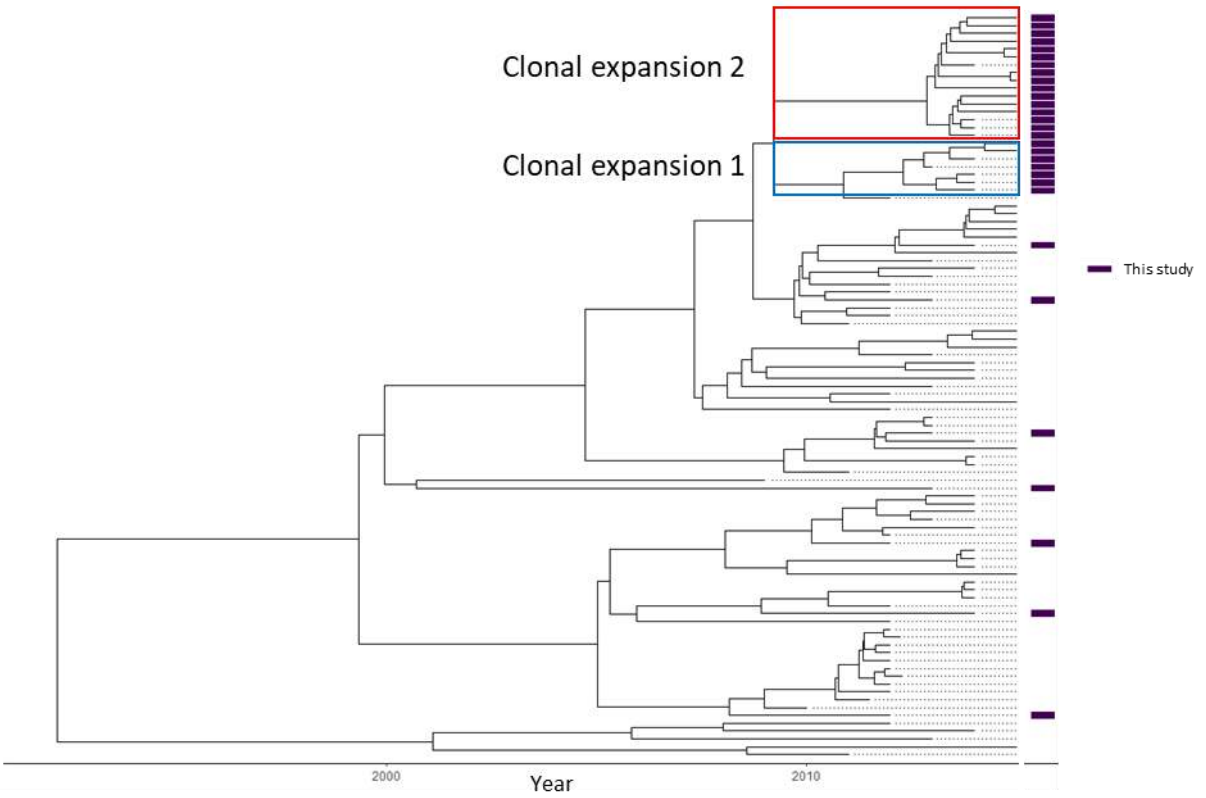


Figure adapted from Wyres *et al.* 2019. Isolates in this study marked with purple bars. Proposed clonal expansion 1 and 2 marked with red and blue box, respectively.

Supplementary table S2 - Distribution of K-loci amonc clonal groups

class	K-locus	n	%	Clonal groups (CGs)
esbl	unknown	53	26,4 %	CG307 (n=9), CG15 (n=5), CG20 (n=4), CG2947 (n=4), CG258 (n=4), CG35 (n=3), CG70 (n=3), CG45 (n=2), CG1393 (n=1), CG25 (n=1), CG251 (n=1), CG26 (n=1), CG268 (n=1), CG27 (n=1), CG29 (n=1), CG2950 (n=1), CG2952 (n=1), CG37 (n=1), CG377 (n=1), CG39 (n=1), CG392 (n=1), CG405 (n=1), CG48 (n=1), CG551 (n=1), CG661 (n=1), CG716 (n=1), CG76 (n=1)
esbl	KL102	27	13,4 %	CG307 (n=25), CG268 (n=2)
esbl	KL2	11	5,5 %	CG15 (n=9), CG35 (n=1), CG416 (n=1)
esbl	KL24	11	5,5 %	CG45 (n=6), CG15 (n=5)
esbl	KL136	10	5,0 %	CG70 (n=10)
esbl	KL38	9	4,5 %	CG261 (n=5), CG37 (n=3), CG873 (n=1)
esbl	KL62	9	4,5 %	CG48 (n=6), CG198 (n=1), CG2552 (n=1), CG45 (n=1)
esbl	KL15	8	4,0 %	CG258 (n=5), CG37 (n=2), CG392 (n=1)
esbl	KL10	4	2,0 %	CG551 (n=2), CG107 (n=1), CG392 (n=1)
esbl	KL17	4	2,0 %	CG101 (n=3), CG322 (n=1)
esbl	KL28	4	2,0 %	CG20 (n=1), CG26 (n=1), CG2948 (n=1), CG661 (n=1)
esbl	KL3	4	2,0 %	CG896 (n=3), CG873 (n=1)
esbl	KL43	4	2,0 %	CG904 (n=4)
esbl	KL51	4	2,0 %	CG2947 (n=3), CG20 (n=1)
esbl	KL112	3	1,5 %	CG15 (n=3)
esbl	KL25	3	1,5 %	CG20 (n=2), CG45 (n=1)
esbl	KL27	3	1,5 %	CG392 (n=3)
esbl	KL48	3	1,5 %	CG15 (n=3)
esbl	KL105	2	1,0 %	CG1296 (n=1), CG258 (n=1)
esbl	KL107	2	1,0 %	CG377 (n=1), CG659 (n=1)
esbl	KL21	2	1,0 %	CG290 (n=1), CG323 (n=1)
esbl	KL46	2	1,0 %	CG2428 (n=1), CG277 (n=1)
esbl	KL5	2	1,0 %	CG686 (n=2)
esbl	KL9	2	1,0 %	CG22 (n=1), CG719 (n=1)
esbl	KL103	1	0,5 %	CG334 (n=1)
esbl	KL108	1	0,5 %	CG2947 (n=1)
esbl	KL13	1	0,5 %	CG485 (n=1)
esbl	KL14	1	0,5 %	CG37 (n=1)
esbl	KL151	1	0,5 %	CG405 (n=1)
esbl	KL19	1	0,5 %	CG2946 (n=1)
esbl	KL20	1	0,5 %	CG133 (n=1)
esbl	KL23	1	0,5 %	CG584 (n=1)
esbl	KL31	1	0,5 %	CG4246 (n=1)
esbl	KL45	1	0,5 %	CG874 (n=1)
esbl	KL53	1	0,5 %	CG489 (n=1)
esbl	KL54	1	0,5 %	CG29 (n=1)
esbl	KL63	1	0,5 %	CG111 (n=1)
esbl	KL64	1	0,5 %	CG392 (n=1)
esbl	KL7	1	0,5 %	CG4 (n=1)

class	K-locus	n	%	Clonal groups (CGs)
non-esbl	unknown	305	45,7 %	CG26 (n=25), CG20 (n=15), CG37 (n=15), CG35 (n=7), CG641 (n=7), CG643 (n=7), CG10 (n=6), CG1562 (n=6), CG359 (n=6), CG4 (n=5), CG925 (n=5), CG1875 (n=4), CG2947 (n=4), CG3084 (n=4), CG461 (n=4), CG105 (n=3), CG240 (n=3), CG25 (n=3), CG251 (n=3), CG268 (n=3), CG27 (n=3), CG29 (n=3), CG4230 (n=3), CG45 (n=3), CG596 (n=3), CG639 (n=3), CG896 (n=3), CG1114 (n=2), CG1142 (n=2), CG1308 (n=2), CG133 (n=2), CG15 (n=2), CG1423 (n=2), CG1456 (n=2), CG1681 (n=2), CG1777 (n=2), CG197 (n=2), CG200 (n=2), CG250 (n=2), CG338 (n=2), CG347 (n=2), CG3919 (n=2), CG416 (n=2), CG4206 (n=2), CG475 (n=2), CG681 (n=2), CG70 (n=2), CG919 (n=2), CG1035 (n=1), CG1096 (n=1), CG111 (n=1), CG1189 (n=1), CG1193 (n=1), CG1317 (n=1), CG134 (n=1), CG1393 (n=1), CG1485 (n=1), CG1609 (n=1), CG1646 (n=1), CG1664 (n=1), CG1727 (n=1), CG1778 (n=1), CG1791 (n=1), CG180 (n=1), CG1845 (n=1), CG188 (n=1), CG189 (n=1), CG1966 (n=1), CG198 (n=1), CG1980 (n=1), CG2010 (n=1), CG209 (n=1), CG215 (n=1), CG23 (n=1), CG2309 (n=1), CG2386 (n=1), CG2421 (n=1), CG2441 (n=1), CG2445 (n=1), CG2459 (n=1), CG2558 (n=1), CG285 (n=1), CG2850 (n=1), CG292 (n=1), CG294 (n=1), CG2942 (n=1), CG2952 (n=1), CG30 (n=1), CG3048 (n=1), CG3083 (n=1), CG3175 (n=1), CG321 (n=1), CG334 (n=1), CG258 (n=1), CG3506 (n=1), CG3565 (n=1), CG363 (n=1), CG378 (n=1), CG384 (n=1), CG392 (n=1), CG4024 (n=1), CG405 (n=1), CG4076 (n=1), CG4148 (n=1), CG4151 (n=1), CG4156 (n=1), CG4157 (n=1), CG4161 (n=1), CG4163 (n=1), CG4164 (n=1), CG4171 (n=1), CG4174 (n=1), CG4177 (n=1), CG4187 (n=1), CG4189 (n=1), CG4199 (n=1), CG4214 (n=1), CG4218 (n=1), CG4223 (n=1), CG4229 (n=1), CG4243 (n=1), CG4249 (n=1), CG4261 (n=1), CG4281 (n=1), CG4328 (n=1), CG4331 (n=1), CG4333 (n=1), CG4340 (n=1), CG4348 (n=1), CG4359 (n=1), CG4360 (n=1), CG4374 (n=1), CG4376 (n=1), CG4378 (n=1), CG4387 (n=1), CG4389 (n=1), CG4393 (n=1), CG4398 (n=1), CG4400 (n=1), CG477 (n=1), CG48 (n=1), CG485 (n=1), CG499 (n=1), CG501 (n=1), CG515 (n=1), CG584 (n=1), CG616 (n=1), CG628 (n=1), CG686 (n=1), CG697 (n=1), CG76 (n=1), CG767 (n=1), CG86 (n=1), CG889 (n=1), CG942 (n=1)
non-esbl	KL28	18	2,7 %	CG20 (n=11), CG26 (n=4), CG15 (n=1), CG2478 (n=1), CG70 (n=1)
non-esbl	KL10	17	2,5 %	CG359 (n=6), CG26 (n=5), CG198 (n=1), CG252 (n=1), CG515 (n=1), CG551 (n=1), CG584 (n=1), CG76 (n=1)
non-esbl	KL2	14	2,1 %	CG25 (n=7), CG15 (n=4), CG86 (n=2), CG20 (n=1)
non-esbl	KL38	14	2,1 %	CG1423 (n=3), CG20 (n=3), CG37 (n=2), CG641 (n=2), CG3147 (n=1), CG318 (n=1), CG258 (n=1), CG584 (n=1)
non-esbl	KL54	12	1,8 %	CG29 (n=6), CG1778 (n=2), CG3231 (n=1), CG4181 (n=1), CG616 (n=1), CG889 (n=1)
non-esbl	KL1	11	1,6 %	CG23 (n=6), CG2159 (n=1), CG249 (n=1), CG4071 (n=1), CG417 (n=1), CG440 (n=1)
non-esbl	KL25	11	1,6 %	CG134 (n=5), CG20 (n=1), CG27 (n=1), CG2850 (n=1), CG4265 (n=1), CG461 (n=1), CG643 (n=1)
non-esbl	KL102	10	1,5 %	CG10 (n=8), CG105 (n=2)
non-esbl	KL24	10	1,5 %	CG15 (n=4), CG359 (n=2), CG45 (n=2), CG20 (n=1), CG27 (n=1)
non-esbl	KL31	10	1,5 %	CG26 (n=2), CG104 (n=1), CG1096 (n=1), CG188 (n=1), CG1984 (n=1), CG2010 (n=1), CG2108 (n=1), CG4192 (n=1), CG4251 (n=1)
non-esbl	KL62	10	1,5 %	CG461 (n=5), CG643 (n=2), CG160 (n=1), CG45 (n=1), CG664 (n=1)
non-esbl	KL21	9	1,3 %	CG37 (n=2), CG1630 (n=1), CG200 (n=1), CG2609 (n=1), CG290 (n=1), CG323 (n=1), CG4217 (n=1), CG499 (n=1)
non-esbl	KL30	9	1,3 %	CG234 (n=2), CG29 (n=2), CG416 (n=2), CG198 (n=1), CG252 (n=1), CG4239 (n=1)
non-esbl	KL63	9	1,3 %	CG111 (n=3), CG3067 (n=2), CG4203 (n=2), CG4225 (n=1), CG4396 (n=1)
non-esbl	KL105	8	1,2 %	CG1114 (n=6), CG639 (n=1), CG774 (n=1)
non-esbl	KL60	7	1,0 %	CG641 (n=3), CG1224 (n=1), CG1915 (n=1), CG3702 (n=1), CG3919 (n=1)
non-esbl	KL103	6	0,9 %	CG107 (n=1), CG1984 (n=1), CG2010 (n=1), CG3975 (n=1), CG4188 (n=1), CG4352 (n=1)
non-esbl	KL134	6	0,9 %	CG515 (n=5), CG4193 (n=1)
non-esbl	KL17	6	0,9 %	CG322 (n=4), CG101 (n=1), CG2947 (n=1)
non-esbl	KL3	6	0,9 %	CG76 (n=2), CG209 (n=1), CG321 (n=1), CG4192 (n=1), CG4203 (n=1)
non-esbl	KL5	6	0,9 %	CG2421 (n=1), CG2559 (n=1), CG290 (n=1), CG4252 (n=1), CG45 (n=1), CG60 (n=1)
non-esbl	KL53	6	0,9 %	CG1875 (n=3), CG363 (n=2), CG475 (n=1)
non-esbl	KL7	6	0,9 %	CG187 (n=2), CG26 (n=2), CG4 (n=2)
non-esbl	KL9	6	0,9 %	CG197 (n=2), CG1317 (n=1), CG22 (n=1), CG584 (n=1), CG628 (n=1)
non-esbl	KL22	5	0,7 %	CG35 (n=2), CG1193 (n=1), CG248 (n=1), CG3639 (n=1)
non-esbl	KL23	5	0,7 %	CG37 (n=2), CG1262 (n=1), CG20 (n=1), CG4176 (n=1)
non-esbl	KL27	5	0,7 %	CG268 (n=2), CG661 (n=2), CG26 (n=1)
non-esbl	KL117	4	0,6 %	CG2947 (n=2), CG4238 (n=1), CG4290 (n=1)
non-esbl	KL13	4	0,6 %	CG189 (n=1), CG251 (n=1), CG33 (n=1), CG4 (n=1)
non-esbl	KL14	4	0,6 %	CG2441 (n=2), CG363 (n=1), CG37 (n=1)
non-esbl	KL143	4	0,6 %	CG214 (n=3), CG4223 (n=1)
non-esbl	KL16	4	0,6 %	CG15 (n=2), CG215 (n=1), CG4203 (n=1)
non-esbl	KL20	4	0,6 %	CG268 (n=1), CG35 (n=1), CG485 (n=1), CG893 (n=1)
non-esbl	KL34	4	0,6 %	CG1791 (n=1), CG312 (n=1), CG347 (n=1), CG697 (n=1)

non-esbl	KL39	4	0,6 %	CG26 (n=1), CG3370 (n=1), CG3878 (n=1), CG4174 (n=1)
non-esbl	KL55	4	0,6 %	CG20 (n=1), CG392 (n=1), CG4382 (n=1), CG475 (n=1)
non-esbl	KL57	4	0,6 %	CG1035 (n=1), CG3955 (n=1), CG4224 (n=1), CG643 (n=1)
non-esbl	KL61	4	0,6 %	CG1845 (n=1), CG240 (n=1), CG4079 (n=1), CG4228 (n=1)
non-esbl	KL114	3	0,4 %	CG1535 (n=1), CG628 (n=1), CG719 (n=1)
non-esbl	KL18	3	0,4 %	CG101 (n=1), CG4384 (n=1), CG967 (n=1)
non-esbl	KL35	3	0,4 %	CG1096 (n=1), CG4154 (n=1), CG4206 (n=1)
non-esbl	KL46	3	0,4 %	CG2428 (n=1), CG277 (n=1), CG4233 (n=1)
non-esbl	KL107	2	0,3 %	CG4174 (n=1), CG4316 (n=1)
non-esbl	KL108	2	0,3 %	CG129 (n=1), CG461 (n=1)
non-esbl	KL11	2	0,3 %	CG4205 (n=1), CG4349 (n=1)
non-esbl	KL110	2	0,3 %	CG268 (n=1), CG258 (n=1)
non-esbl	KL112	2	0,3 %	CG15 (n=1), CG240 (n=1)
non-esbl	KL113	2	0,3 %	CG184 (n=1), CG925 (n=1)
non-esbl	KL116	2	0,3 %	CG133 (n=2)
non-esbl	KL12	2	0,3 %	CG200 (n=1), CG37 (n=1)
non-esbl	KL122	2	0,3 %	CG10 (n=1), CG70 (n=1)
non-esbl	KL124	2	0,3 %	CG4349 (n=1), CG48 (n=1)
non-esbl	KL128	2	0,3 %	CG189 (n=1), CG4035 (n=1)
non-esbl	KL136	2	0,3 %	CG37 (n=1), CG515 (n=1)
non-esbl	KL142	2	0,3 %	CG26 (n=2)
non-esbl	KL149	2	0,3 %	CG105 (n=1), CG2738 (n=1)
non-esbl	KL158	2	0,3 %	CG397 (n=1), CG776 (n=1)
non-esbl	KL48	2	0,3 %	CG4206 (n=1), CG686 (n=1)
non-esbl	KL52	2	0,3 %	CG501 (n=1), CG873 (n=1)
non-esbl	KL64	2	0,3 %	CG30 (n=1), CG4395 (n=1)
non-esbl	KL67	2	0,3 %	CG1562 (n=1), CG4368 (n=1)
non-esbl	KL101	1	0,1 %	CG767 (n=1)
non-esbl	KL111	1	0,1 %	CG1777 (n=1)
non-esbl	KL118	1	0,1 %	CG322 (n=1)
non-esbl	KL120	1	0,1 %	CG1407 (n=1)
non-esbl	KL121	1	0,1 %	CG107 (n=1)
non-esbl	KL123	1	0,1 %	CG200 (n=1)
non-esbl	KL125	1	0,1 %	CG919 (n=1)
non-esbl	KL127	1	0,1 %	CG440 (n=1)
non-esbl	KL130	1	0,1 %	CG1562 (n=1)
non-esbl	KL137	1	0,1 %	CG4371 (n=1)
non-esbl	KL145	1	0,1 %	CG70 (n=1)
non-esbl	KL147	1	0,1 %	CG20 (n=1)
non-esbl	KL162	1	0,1 %	CG248 (n=1)
non-esbl	KL166	1	0,1 %	CG719 (n=1)
non-esbl	KL19	1	0,1 %	CG1562 (n=1)
non-esbl	KL33	1	0,1 %	CG499 (n=1)
non-esbl	KL45	1	0,1 %	CG4234 (n=1)
non-esbl	KL47	1	0,1 %	CG1562 (n=1)
non-esbl	KL49	1	0,1 %	CG1142 (n=1)
non-esbl	KL56	1	0,1 %	CG475 (n=1)
non-esbl	KL58	1	0,1 %	CG3878 (n=1)
non-esbl	KL6	1	0,1 %	CG4271 (n=1)
non-esbl	KL71	1	0,1 %	CG3938 (n=1)
non-esbl	KL74	1	0,1 %	CG392 (n=1)
non-esbl	KL8	1	0,1 %	CG453 (n=1)
non-esbl	KL81	1	0,1 %	CG252 (n=1)

Supplementary table S3 - Distribution of virulence loci

	Species	Hypervirulence[1]	Bla_ESBL_acquired	ST	CG	K_locus	Kleborate virulence_score	Yersiniabactin	Colibactin	Aerobactin	Salmochelin	RmpADC	rmpA2
ESBL group	*Klebsiella pneumoniae sensu stricto*	+	CTX-M-15;SHV-5	ST15	CG15	KL24	4	ybt 13; ICEKp2	-	iuc 1	-	-	rmpA2_8*
	Klebsiella pneumoniae sensu stricto	+	CTX-M-15	ST15	CG15	KL24	4	ybt 13; ICEKp2	-	iuc 1	-	-	rmpA2_8*
	Klebsiella pneumoniae sensu stricto		CTX-M-1	ST2954	CG45	KL24	4	ybt 10; ICEKp4	-	iuc 3	-	-	-
	Klebsiella pneumoniae sensu stricto	+	CTX-M-15	ST420	CG133	KL20	4	ybt 9; ICEKp3	-	iuc 1	iro 1	rmp 1; KpVP-1	-
Non-ESBL group	*Klebsiella pneumoniae sensu stricto*	+	-	ST23	CG23	unknown (best match = KL1)	5	ybt 1; ICEKp10	clb 2	iuc 1	iro 1	-	-
	Klebsiella pneumoniae sensu stricto	+	-	ST23	CG23	KL1	5	ybt 1; ICEKp10	clb 2	iuc 1	iro 1	rmp 1; KpVP-1	-
	Klebsiella pneumoniae sensu stricto	+	-	ST23	CG23	KL1	5	ybt 1; ICEKp10	clb 2	iuc 1	iro 1	rmp 1; KpVP-1	-
	Klebsiella pneumoniae sensu stricto	+	-	ST23	CG23	KL1	5	ybt 1; ICEKp10	clb 2	iuc 1	iro 1	1; KpVP-1 (incomp	-
	Klebsiella pneumoniae sensu stricto	+	-	ST23	CG23	KL1	5	ybt 1; ICEKp10	clb 2	iuc 1	iro 1	rmp 1; KpVP-1	-
	Klebsiella pneumoniae sensu stricto	+	-	ST23	CG23	KL1	5	ybt 1; ICEKp10	clb 2	iuc 1	iro 1	rmp 1; KpVP-1	-
	Klebsiella pneumoniae sensu stricto	+	-	ST23	CG23	KL1	5	ybt 1; ICEKp10	clb 2	iuc 1	iro 1	rmp 1; KpVP-1	-
	Klebsiella pneumoniae sensu stricto	+	-	ST417	CG417	KL1	5	ybt 12; ICEKp10	clb 1	iuc unknown	iro 1	rmp 1; KpVP-1	-
	Klebsiella pneumoniae sensu stricto	+	-	ST592	CG643	KL57	5	ybt 0; ICEKp10	clb 3	iuc 1	iro 1	rmp 1; KpVP-1	-
	Klebsiella pneumoniae sensu stricto		-	ST881	CG25	KL2	4	ybt 16; ICEKp12	-	iuc 3	-	-	-
	Klebsiella pneumoniae sensu stricto	+	-	ST893	CG893	KL20	4	ybt unknown	-	iuc 1	iro 1	rmp 1; KpVP-1	rmpA2_8*
	Klebsiella pneumoniae sensu stricto		-	ST2948	CG2948	KL28	3	-	-	iuc 3	-	-	-
	Klebsiella pneumoniae sensu stricto	+	-	ST592	CG643	unknown (best match = KL50)	3	-	-	iuc 1	iro 1	rmp 1; KpVP-1	-
	Klebsiella pneumoniae sensu stricto	+	-	ST592	CG643	unknown (best match = KL50)	3	-	-	iuc 1	iro 1	1; KpVP-1 (incomp	-
	Klebsiella pneumoniae sensu stricto	+	-	ST86	CG86	KL2	3	-	-	iuc 1	iro 1	rmp 1; KpVP-1	-
	Klebsiella pneumoniae sensu stricto	+	-	ST86	CG86	unknown (best match = KL2)	3	-	-	iuc 1	iro 1	rmp 1; KpVP-1	-
	Klebsiella pneumoniae sensu stricto	+	-	ST86	CG86	KL2	3	-	-	iuc 1	iro 1	rmp 1; KpVP-1	-
	Klebsiella pneumoniae sensu stricto		-	ST133	CG133	KL116	2	ybt 17; ICEKp10	clb 3	-	-	-	-
	Klebsiella pneumoniae sensu stricto		-	ST133	CG133	KL116	2	ybt 17; ICEKp10	clb 3	-	-	-	-

1. HV was defined as either a) the presence of rmpA or rmpA2; and/or b) the presence of aerobactin (iuc) and salmochelin (iro)

Supplementary table S4 - Replicon distribution

			Replicon family																		
	species	n	Col	IncA	IncB	IncC	IncFIA	IncFIB	IncFII	IncH	IncI	IncL	IncM	IncN	IncQ	IncR	IncU	IncX	IncY	rep_a	pKP1433
ESBL	total	201	62.7 % (n=126)	2.0 % (n=4)	1.5 % (n=3)	2.0 % (n=4)	6.0 % (n=12)	87.6 % (n=176)	82.6 % (n=166)	6.5 % (n=13)	1.5 % (n=3)	1.5 % (n=3)	1.5 % (n=3)	4.5 % (n=9)	6.0 % (n=12)	18.9 % (n=38)		2.0 % (n=4)	0.5 % (n=1)		
ESBL	*Klebsiella pneumon*	198	63.1 % (n=125)	2.0 % (n=4)	1.5 % (n=3)	2.0 % (n=4)	6.1 % (n=12)	87.9 % (n=174)	82.8 % (n=164)	6.1 % (n=12)	1.5 % (n=3)	1.0 % (n=2)	1.0 % (n=2)	4.5 % (n=9)	6.1 % (n=12)	18.2 % (n=36)		2.0 % (n=4)	0.5 % (n=1)		
ESBL	*Klebsiella quasipneumoniae* ssp. *similipneumoniae*	2	50.0 % (n=1)					100.0 % (n=2)	50.0 % (n=1)	50.0 % (n=1)		50.0 % (n=1)	50.0 % (n=1)			50.0 % (n=1)					
ESBL	*Klebsiella variicola*	1							100.0 % (n=1)							100.0 % (n=1)					
Non-ESBL	total	667	58.9 % (n=393)		0.1 % (n=1)		15.0 % (n=100)	72.0 % (n=480)	48.7 % (n=325)	5.7 % (n=38)	0.3 % (n=2)	0.6 % (n=4)	0.7 % (n=5)	3.0 % (n=20)	0.3 % (n=2)	14.5 % (n=97)	0.1 % (n=1)			0.9 % (n=6)	0.1 % (n=1)
Non-ESBL	*Klebsiella pneumon*	461	63.3 % (n=292)		0.2 % (n=1)		14.3 % (n=66)	79.4 % (n=366)	54.2 % (n=250)	7.6 % (n=35)	0.4 % (n=2)	0.7 % (n=3)	0.9 % (n=4)	3.5 % (n=16)	0.4 % (n=2)	14.1 % (n=65)	0.2 % (n=1)			0.9 % (n=4)	0.2 % (n=1)
Non-ESBL	*Klebsiella quasipneumoniae* ssp. *quasipneumoniae*	21	47.6 % (n=10)				9.5 % (n=2)	33.3 % (n=7)	14.3 % (n=3)			4.8 % (n=1)	4.8 % (n=1)	9.5 % (n=2)		14.3 % (n=3)					
Non-ESBL	*Klebsiella quasipneumoniae* ssp. *similipneumoniae*	22	59.1 % (n=13)				13.6 % (n=3)	54.5 % (n=12)	27.3 % (n=6)							40.9 % (n=9)					
Non-ESBL	*Klebsiella variicola* ssp. *variicola*	163	47.9 % (n=78)				17.8 % (n=29)	58.3 % (n=95)	40.5 % (n=66)	1.8 % (n=3)				1.2 % (n=2)		12.3 % (n=20)				1.2 % (n=2)	

Supplementary table S5- Temporal and geographical distribution, and SNP ranges in prevalent CGs

		SNPs difference within clonal group						
Clonal group (CG)	n	min	max	median	IQR	Year first detected	Found at least once during 2001-2015 in regions:	Sequence types(STs) within clonal group
CG15	39	0	12439	8806	9667	2001	East, Middle, North, Oslo_Akershus, South, West	ST14, ST15, ST627
CG20	43	0	14379	6930	8509	2001	East, Middle, North, Oslo_Akershus, South, West	ST1412, ST16, ST17, ST20, ST22, ST336, ST422, ST4285, ST636
CG26	44	0	20488	7564	3317.75	2001	East, Middle, North, Oslo_Akershus, South, West	ST163, ST2116, ST2211, ST253, ST26, ST3043, ST3191, ST4273, ST4279, ST4383, ST704
CG70	18	0	14694	52.5	12829	2001	East, Middle, Oslo_Akershus, South, West	ST1873, ST2344, ST4283, ST70
CG307	34	0	109	39	43	2012	East, Middle, North, Oslo_Akershus, South, West	ST307
CG48	9	0	21449	166	2122	2001	East, Middle, Oslo_Akershus, West	ST1411, ST48
CG45	17	0	11319	2885	3032	2005	East, Middle, Oslo_Akershus, South, West	ST2954, ST45, ST485
CG258	13	0	12451	3559	7295	2009	East, Middle, North, Oslo_Akershus, South, West	ST11, ST340, ST437

Supplementary methods

Whole genome sequencing

All study isolates were subject to short-read WGS. DNA was extracted using the MagNAPure 96 system (Roche Applied Science, Manheim, Germany) and sequencing libraries were prepared according to the Nextera XT DNA Library preparation protocol (Illumina, San Diego, CA, USA). WGS was performed on an Illumina MiSeq platform with v3 chemistry to generate 2x300 bp paired-end reads. For isolates where only FASTQ files were received, sequencing had been performed on an Illumina HiSeq 2500 platform at Eurofins Genomics (Eurofins Genomics Europe, Konstanz, Germany) generating 2x125 bp paired-end reads.

To achieve closed genomes for selected isolates, additional long read WGS was performed. DNA was extracted manually using the Beckman Coulter Life Science GenFind V3 kit (C34881) according to the supplemental protocol 'DNA extraction from Bacteria using GenFind v3' (Beckman Coulter, Brea, CA, USA). DNA libraries were prepared using the 1D Ligation sequencing kit (SQK-LSK108) and the Native barcoding kit (EXP-NBD103) (Oxford Nanopore Technologies [ONT], Oxford, United Kingdom) according to the ONT protocol 'native barcoding genomic DNA' or 'genomic DNA by ligation' without shearing to maximize the sequencing read length. Finally, libraries were loaded onto a R9.4.1 MinION flow cell (FLO-MIN106) or a R9.4.1 Flongle flow cell (FLO-FLG001) and sequenced on the ONT MinION Mk1B device (MIN-101B).

In-silico analyses

Short-read sequences were trimmed based on quality and adapter content with TrimGalore v0.5.0 [1] and *de novo* assembly was performed with Unicycler v0.4.8 [2], which uses SPAdes v3.13.1 [3] for assembly and Pilon v1.23 [4] for polishing. Kleborate v2.0.4[5] was used to identify species and determine of multilocus sequence type (ST), virulence loci and AMR genes (CARD database v3.0.8 [6]) from assembled genomes.). Kaptive [7] was used to identify capsule (K) biosynthesis loci reporting

calls with confidence level "Good" or higher. Putative ESBLs with no definite ESBL-gene had their read sets investigated using SRST2 v.2.0.4[8] with CARD database v3.0.8, as some reads may have been discarded in the assembly process. Only ESBL-gene matches with 100 % sequence coverage and identity were included in further analyses. Plasmid replicons were identified with SRST2 v0.2.0 using the Plasmidfinder database version 2021-01-13. [9]

A core chromosomal single-nucleotide variant (SNV) alignment of the verified ESBL and non-ESBL genomes was generated to assess their relatedness. The short-reads were mapped to the chromosome of the ST23 reference genome NTUH-K2044 (NC_01273.1) with the RedDog V1beta.11 [10] pipeline, using Bowtie2 v2.3.4.2[11] for read mapping and SAMtools v1.9[12] for SNV calling. RedDog was used with default parameters as described previously [13], except for the read depth threshold which was set to ≥8 (default ≥10) to include all genomes. A maximum likelihood (ML) phylogeny was inferred from the resulting alignment (868 genomes, 867815 SNPs) using FastTree v2.1.10 (gamma distribution of rate heterogeneity among sites)[14].

Clonal groups (CGs) were defined by patristic distances. This method was chosen as it has previously been used to cluster CGs in KpSC (Bialek-Davenet, S., et al., 2019) and a distance threshold of 0.04 was used as it grouped STs that have previously been identified as belonging to clinically distinct CGs. The CGs dominated by ST14 and ST340 where denoted CG15 and CG258, as these names are more commonly used. [15, 16].

Long-read sequences were base called in barcode-trimmed and de-multiplexed high-accuracy mode using Guppy Basecalling Software v3.2.4+d9ed22f[17161716][16][16][16][16](16), followed by quality filtering with Filtlong v0.2.0[18]. To resolve the complete genome sequence of these isolates, hybrid assembly with the corresponding short-read genomes using Unicycler v0.4.8 was performed. The

completed genomes were subsequently annotated with the NCBI Prokaryotic Annotation Pipeline v5.1 [19], using default parameters.

To assess the clonal relatedness of the ST307 genomes, an alignment was generated with RedDog, using the hybrid-assembled closed ST307 genome as the mapping reference . The resulting alignment with 391 variant sites was screened for recombination events using Gubbins v2.3.4 [20] with convergence method "weighted Robinson-Foulds". This alignment was passed to RAxML v8.2.10[21] to infer ML phylogeny. The best-scoring ML tree was chosen from five independent runs with the GTR+ nucleotide substitution model, followed by a rapid bootstrap analysis (100 replicates) to estimate branch support.

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