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Genomic characterization of ESBL/AmpC-producing and high-risk clonal lineages of *Escherichia coli* and *Klebsiella pneumoniae* in imported dogs with shelter and stray background

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ABSTRACT

Objectives: Extended spectrum β-lactamase (ESBL)- and ampicillinase C (AmpC)-carrying Enterobacteriaceae have been widely reported among companion animals. According to previous studies, dogs with a shelter or stray background might be at risk of carrying such bacteria. The aim of this study was to explore, with whole-genome sequencing (WGS), the genomic characteristics of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from imported dogs with a stray or shelter background.
Methods: *E. coli* (n = 58) and *K. pneumoniae* (n = 2) isolates from imported dogs originating from seven countries were included. Phenotypic resistance was investigated by selective isolation and antibiotic susceptibility testing. Whole-genome sequencing was used to study the genomic characteristics and the presence of antimicrobial resistance genes (ARGs) and virulence determinants of the ESBL/AmpC-producing *E. coli* and *K. pneumoniae* isolates.
Results: A high diversity of different ARGs (n = 56) and sequence types (STs) (n = 32), including high-risk clonal lineages ST410 (n = 3) and ST307 (n = 1), was identified in *E. coli* and *K. pneumoniae* isolates, respectively. Genes encoding resistance to β-lactams accounted for the majority, with the most frequent being *blaCTX-M-15*. Moreover, 17 (29%) *E. coli* isolates qualified as presumptive extraintestinal pathogenic and/or uropathogenic *E. coli*.
Conclusions: Our results highlight the multiplicity of genetic backgrounds disseminating ESBL/AmpC-genes in the studied dogs, calling for further investigation of possible drivers responsible for the dissemination of ARGs in animal shelters and amongst stray dogs. From a public health perspective, enhanced genomic surveillance of ESBL/AmpC-producing Enterobacteriaceae in dogs is needed in Finland.

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1. Introduction

Recently, extended spectrum β-lactamase (ESBL)- and ampicillinase C (AmpC)-carrying Enterobacteriaceae have been widely reported in companion animals worldwide. Within the Enterobacteriaceae family, the emergence of certain ESBL- and plasmid-mediated AmpC-encoding genes conferring resistance to broad spectrum cephalosporins are often attributable to mobile genetic elements. Amongst these, *blaCTX-M-15* has been reported as a predominant β-lactamase gene amongst humans and companion animals due to successful *Escherichia coli* clones and plasmids [1,2]. In addition to *blaCTX-M-15*, a high diversity of different ESBL genes and *E. coli* clones has been reported within dogs and cats, indicating multiple potential sources or a selective pressure generated by antibiotics [2].

The estimated global prevalence of ESBL-producing *E. coli* in dogs is 6.29% [2], and earlier studies from Brazil, Romania, and Japan have shown considerably higher proportions of ESBL-producing Enterobacteriaceae in sheltered and stray dogs, ranging from 14.6 to 31.0% [3–5]. Moreover, a recent project by the Finnish Food Authority revealed that the proportion of ESBL-producing En-

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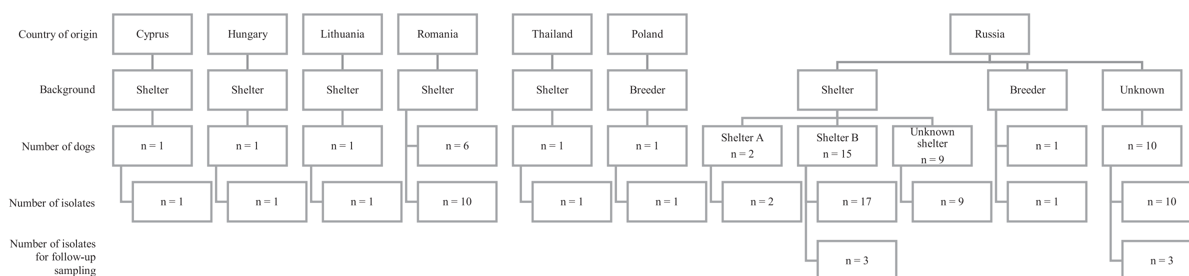

Fig. 1.

terobacteriaceae was 30% amongst imported dogs, many of which had a stray or shelter background [6]. Notably, the proportion of ESBL-producing Enterobacteriaceae was even higher for dogs originating from Russia (55%) and Romania (43%) [6], while the estimated carriage of ESBL/AmpC-producing *E. coli* in Finnish dogs and humans is estimated to be 5% and 6.3%, respectively [7,11]. The number of commercially imported dogs by animal charities and private persons from Russia and Romania has been remarkably high within the last 10 years, including 3503 and 3350 dogs, respectively, between 2013 and 2018. In most cases, these imported adult dogs originate from shelters with a stray background [6].

Indeed, dogs originating from shelters or breeders have been identified to be three times more likely to carry ESBL- or AmpC-producing *E. coli* compared with dogs from private owners [8], and a higher level of resistance in *E. coli* strains from stray dogs compared with dogs with permanent homes has also been reported [9]. Potential risk factors for ESBL/AmpC-carriage in stray dogs are free movement in large areas, scavenging of food remains and human/animal waste, lack of sanitation, and exposure to the environmental resistome [9,10]. In addition, nosocomial dissemination of ESBL-producing Enterobacteriaceae has been suggested to occur in shelter environments due to insufficient hygienic practises [3].

The molecular description of ESBL/AmpC-producing Enterobacteriaceae in companion animals remains scarce in some countries, and little information is available in Finland [11,12]. Although household transmission between pet owners and pets seems to occur sporadically [12–14], the high proportion of ESBL/AmpC-carrying Enterobacteriaceae isolates in imported dogs could play a bridging role in the circulation of such bacteria between humans and companion animals. In this study, we used whole-genome sequencing (WGS) to characterize the genomes of the ESBL/AmpC-producing Enterobacteriaceae isolated from dogs either imported or intended to be imported to Finland to elaborate the genetic backgrounds responsible for the high proportion of such bacteria, and to give future directions for mitigation of this issue.

2. Materials and methods

2.1. Sample collection

The material consisted of 60 putative ESBL/AmpC-producing *E. coli* (n = 58) and *Klebsiella pneumoniae* (n = 2) isolates that were selected from the strain collection of the Finnish Food Authority. Isolates had been obtained through the screening of ESBL/AmpC/carbapenemase-producing bacteria from dogs either imported or intended to be imported into Finland during 2017 and 2018. Isolates originated from 48 dogs; 13 dogs had been sampled upon arrival at the airport, 9 dogs at animal clinics within one month of importation, and 18 dogs were sampled in a shelter located in Russia. The rest of the samples were taken from dogs (n = 8) originating from shelters, but already living in Finland for >6 weeks to 6 months. The studied dogs originated from Russia (n = 37), Romania (n = 6), Cyprus (n = 1), Thailand (n = 1), Lithuania (n = 1), Hungary (n = 1), and Poland (n = 1). Most of the studied dogs originated from animal shelters (n = 36) with a possible stray background. The study group is illustrated in Fig. 1. Isolates were obtained from faecal swab samples taken with sterile swabs (M40 Transystem Amies Agar Gel [Copan Diagnostics, Brescia, Italy]) directly from the rectum or fresh faeces (one swab per animal per sampling point). In addition, follow-up samples from three Russian dogs were included to study the duration of the faecal carriage of ESBL-producing Enterobacteriaceae. The follow-up samples were taken 4 to 6 months after the initial sampling. The samples were transported to the laboratory of the Finnish Food Authority's microbiology unit, where they were analysed. Subsequently, isolates were subjected to WGS.

Fig. 1 presents a flow chart showing the origin and number of selected samples and isolates. The total number of Romanian isolates includes three duplicates originating from the same sample/dogs, resulting from a species confirmation step where up to three typical isolates were picked from blood agar plates. Two of the dogs originating from Shelter B carried both AmpC- and ESBL-producing E. coli isolates. Both phenotypes were selected from the same sample/dog for WGS.

2.2. ESBL/AmpC/carbapenemase screening and species identification

The screening of ESBL/AmpC- and carbapenemase-producing *E. coli* was done according to the EU Reference Laboratory for antimicrobial resistance (EURL-AR) protocol [15] with slight modifications. Briefly, swab samples were suspended in 3 mL of buffered peptone water (Merck, Germany) and enriched overnight at 37°C. Subsequently, 10 μL of the enrichment was spread onto MacConkey agar plates (Becton Dickinson and Company, France) supplemented with 1 mg/l cefotaxime (Sigma-Aldrich, Germany) for the detection of presumptive ESBL/AmpC producers, and 10 μL was spread onto CHROMID CARBA and CHROMID OXA-48 agar plates (bioMérieux, France) for the detection of presumptive carbapenemase producers. The MacConkey plates were incubated overnight at 44°C, and the CARBA and OXA-48 plates overnight at 37°C. Up to three typical *E. coli* colonies were picked from the selective plates, subcultured onto blood agar plates, and confirmed with MALDI-TOF (MALDI Biotyper, Bruker Daltonics, Germany) with a score value of ≥2. If typical *E. coli* colonies were not present, other colonies were picked randomly and bacterial species confirmed with MALDI-TOF.

2.3. Susceptibility of presumptive ESBL/AmpC/carbapenemase-producing E. coli and K. pneumoniae isolates

Sensititre EUVSEC and EUVSEC2 (TREK Diagnostic Systems, UK) plates were used for the determination of minimum inhibitory concentration (MIC) values of the presumptive ESBL/AmpC *E. coli* (n = 58) and *Klebsiella* Spp. (n = 2) isolates for the following antimicrobials: ampicillin, azithromycin, ceftazidime/clavulanic

acid, ceftazidime, cefoxitin, chloramphenicol, ciprofloxacin, colistin, cefotaxime/clavulanic acid, cefotaxime, cefepime, gentamicin, ertapenem, imipenem, meropenem, nalidixic acid, sulphamethoxazole, tetracycline, tigecycline, trimethoprim, and temocillin. The epidemiological cut-off (ECOFF) values were used to separate the wild-type population (referred to as susceptible) from non-wild–type isolates (referred to as resistant) [16]. Extended spectrum ß lactamase phenotype was determined by analysing the synergy with cefotaxime/ceftazidime and clavulanic acid. Furthermore, an isolate was phenotypically categorized as AmpC if the cefoxitin MIC was above the ECOFF value. *E. coli* ATCC 25922 was used as the quality control strain.

2.4. DNA extraction and whole-genome sequencing

Bacterial DNA extraction and purification was done for ESBL/AmpC-producing *E. coli* (n = 58) and *K. pneumoniae* (n = 2) isolates with a QIAGEN DNeasy Blood & Tissue Kit (Qiagen, Germany) according to the manufacturer's instructions. DNA quality was assessed with a DeNovix spectrophotometer (DeNovix Inc., Wilmington, DE) and quantity with a Qubit Fluorometer (ThermoFisher Scientific, Malaysia). Library preparation was done using a Nextera DNA Flex Library Prep Kit (Illumina, San Diego, CA) and paired-end sequencing (250 bp read length) was conducted on a MiSeq platform (Illumina, San Diego, CA,). Raw reads have been deposited at EMBL's European Bioinformatics Institute(EMBL-EBI) under accession number PRJEB50707. Accession numbers are provided in Table S1.

2.5. Sequence analyses

Sequenced raw reads were processed using default parameters of the Ridom SeqSphere+ version 7.0.4 bioinformatic pipeline mode, which integrates quality assessment using FastQC version 0.1.1.7 [17], adapter trimming using Trimmomatic version 0.36 [18], and de novo assembly using SKESA version 2.3.0 [19]. Bioinformatic analyses were run on a web-based service (Center for Genomic Epidemiology, DTU, Denmark) using assembled sequences. Identity percentage threshold (95%), minimum length (60%), and minimum coverage (60%) were selected according to the server's default parameters. Antimicrobial resistance genes (ARGs) were determined using ResFinder version 4.1 [20–22], multilocus sequence typing (MLST) was determined with MLST version 2.0 [23] using *E. coli* schemes 1 [24] and 2 [25], virulence genes were determined with VirulenceFinder version 2.0 [26,27], the profile of plasmid replicons and plasmid incompatibility groups was assessed with PlasmidFinder version 2.1 [21,28] and pMLST version 2.0 [21,29], and fimH subtype was determined with FimTyper version 1.0 [30]. *Escherichia* genus strain phylotypes were determined with the ClermonTyping tool [31]. Capsular type prediction, virulence loci prediction, and additional virulence profiling (yersiniabactin, colibactin, aerobactin, and salmochelin clusters) were done for assembled *K. pneumoniae* sequences using Kleborate version 2.0.4 [32] through the epi online platform PathogenWatch [33].

3. Results and discussion

3.1. Species identification and phenotypes of the studied E. coli and K. pneumoniae

Altogether, 57 isolates were confirmed as *E. coli* and two isolates as *K. pneumoniae*. One isolate (FIAR-611) received score values of >2.2 for both *Escherichia albertii* and *E. coli* but was treated as the latter in WGS analyses. Phenotypic ESBL production was detected in 78% (45/58) of the studied *E. coli* isolates, whereas AmpC production was detected in 19% (11/58). Concurrent ESBL and AmpC production (i.e. synergy with third-generation cephalosporin and clavulanic acid) and cefoxitin resistance was detected in 3% (2/58) of the studied *E. coli* isolates. The studied *K. pneumoniae* isolates were both ESBL producers.

3.2. Antimicrobial susceptibility profiles of ESBL/AmpC-producing E. coli

All isolates (n = 58) were resistant to ampicillin, ceftazidime, and cefotaxime. Resistance was commonly observed to fourth-generation cephalosporin cefepime (n = 53; 92.4%), ciprofloxacin (n = 48; 83%), trimethoprim (n = 32; 54.7%), nalidixic acid (n = 25; 43.4%), and tetracycline (n = 24; 41.5%). Other resistance profiles included azithromycin (n = 13.1; 22.6%), ertapenem (n = 12; 20.7%), chloramphenicol (n = 11; 18.9%), gentamicin (n = 6; 11.3%), temocillin (n = 3; 5.7%), and colistin (n = 3; 5.7%). All strains subjected to broth microdilution testing were susceptible to imipenem, meropenem, and tigecycline. The ECOFF value was not available for sulphamethoxazole. The distribution of MICs for all *E. coli* isolates is presented in Table S2.

3.3. Antimicrobial susceptibility profiles of ESBL/AmpC-producing K. pneumoniae isolates

K. pneumoniae isolates (n = 2) were resistant to third-generation cephalosporins (ceftazidime and cefotaxime), cefepime, ciprofloxacin, tigecycline, trimethoprim, and temocillin. Both isolates were susceptible to cefoxitin, colistin, gentamicin, imipenem, meropenem, and tetracycline. Epidemiological cut-off values were not available for ampicillin, azithromycin, ceftazidime/clavulanic acid, chloramphenicol, cefotaxime/clavulanic acid, ertapenem, nalidixic acid, and sulphamethoxazole. The distribution of MICs for all *K. pneumoniae* isolates is presented in Table S3.

3.4. Antimicrobial resistance genes

This study identified 56 different acquired ARGs from the sequenced *E. coli* and *K. pneumoniae* isolates. Genes encoding resistance to β-lactams accounted for the majority with 13 different determinants (Fig. 2). The highest prevalence was for the *blaCTX-M-15* gene, which was found in 36 (60%) of the studied isolates, followed by *blaTEM-1B*, which was found in 25 (42%). There was good agreement between the ESBL/AmpC phenotype and the predicted genotype. All 11 phenotypically categorized AmpC-producing *E. coli* isolates carried an AmpC β-lactamase *blaCMY-2* or *blaCMY-4* gene. Additionally, of these isolates, nine had a *blaTEM-1B* gene. All phenotypically categorized ESBL-producing *E. coli* (n = 45) and *K. pneumoniae* (n = 2) isolates had at least one *blaCTX-M* gene. Two isolates with both ESBL and AmpC phenotypes had a combination of AmpC β-lactamase *blaCMY-2* and ESBL β-lactamase *blaCTX-M-15* genes. In addition to β-lactamases, genes encoding resistance to macrolides, lincosamides and streptogramins (MLS), fluoroquinolones, aminoglycosides, sulphonamides, trimethoprim, and tetracyclines were commonly found (Table 1). Genes conferring resistance to MLS were found in 54 (90%) isolates. Plasmid-mediated quinolone resistance (PMQR) determinants were found in 46 (77%) isolates. In addition to PMQR determinants, mutations in quinolone resistance-determining regions (QRDRs) in the *gyrA* (p.S83L and p.D87N), *parC* (p.S80I and p.A56T), and *parE* (p.S458A) genes were found in 11 (18%) of the studied *E. coli* isolates. Genes encoding resistance to aminoglycosides were found in 34 (57%) of the isolates. Other important ARGs, such as sulphonamide resistance, trimethoprim resistance, tetracycline resistance, phenicol resistance, rifampicin resistance, and plasmid-mediated colistin resistance, were also identified (Table 1).

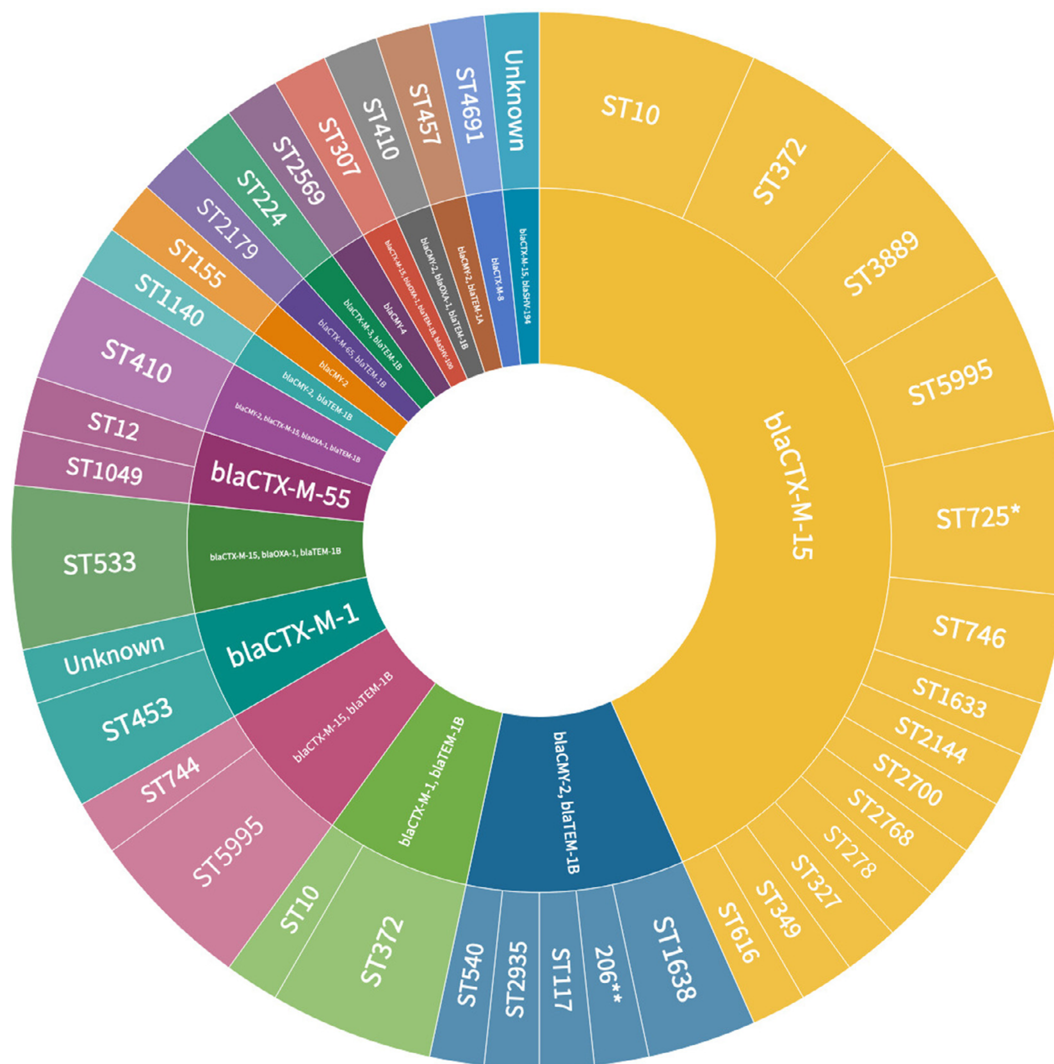

Fig. 2. Distribution of β-lactamase genes and sequence types (STs) in the studied Escherichia coli isolates.
*New *purA* allele, ST may indicate nearest ST.
**New *gyrB* allele, ST may indicate nearest ST.

3.5. Multilocus sequence types

In silico-based MLST analysis was done using seven housekeeping genes. The *E. coli* isolates (n = 58) belonged to 32 different sequence types (STs), the most prevalent being ST372 (n = 6), ST5995 (n = 6), and ST10 (n = 5), followed by ST3889, ST410, ST533, ST725 (n = 3 of each), ST453, ST1638, and ST746 (n = 2 of each). Other STs were found once (Fig. 2). One *K. pneumoniae* isolate belonged to ST307. The other *K. pneumoniae* isolate had an unknown ST.

3.6. Plasmid replicons

Altogether, 34 different plasmid replicons were detected in the studied *E. coli* (n = 58) and *K. pneumoniae* (n = 2) isolates, with the following 11 different incompatibility groups: IncF, IncI, IncK, IncHI, IncN, IncM, IncR, IncQ, IncX1, IncX4, and IncY; Col-like plasmids were also detected. The most frequent incompatibility group by far was the IncF group, including variants IncFll, IncFIA, IncFIB, and IncFIC. The IncFll plasmid replicon was detected in 77% of the isolates (n = 46). The second most frequent incompatibility groups were the IncI group and the IncX group. On average, each isolate carried three different plasmid replicons, ranging from one to seven plasmid replicons.

3.7. Virulence genotyping and phylogroups of ESBL/AmpC-producing *E. coli*

Altogether, 93 different putative virulence-associated genes were detected within the studied isolates (n = 58), and all the studied isolates carried between 2 and 30 extraintestinal pathogenic *E. coli* (ExPEC) virulence-associated genes. Most of the studied *E. coli* isolates belonged to commensal-associated phylotypes (A, 24; B1, 14; C, 3). The rest of the isolates belonged to typical ExPEC phylogroups (B2, 10; D, 1; E, 3; F, 2). The distribution of virulence genes and Clermont phylotypes is presented in Fig. 3. Of the 58 ESBL/AmpC-producing *E. coli* isolates, 17 (29%) qualified as presumptive ExPEC and/or uropathogenic *E. coli* (UPEC), according to previously reported classification definitions [34,35]. Most (n = 10; 56%) of these isolates belonged to phylogroup B2. Moreover, six (33%) of the presumptive ExPEC and/or UPEC isolates belonged to strain ST372. Enteropathogenic *E. coli* associated genes, intimin (*eae*) and translocated intimin receptor (*Tir*), were detected in 12% (n = 7) of the studied isolates. Moreover, the enteroaggregative *E. coli*-associated gene, transcriptional regulator

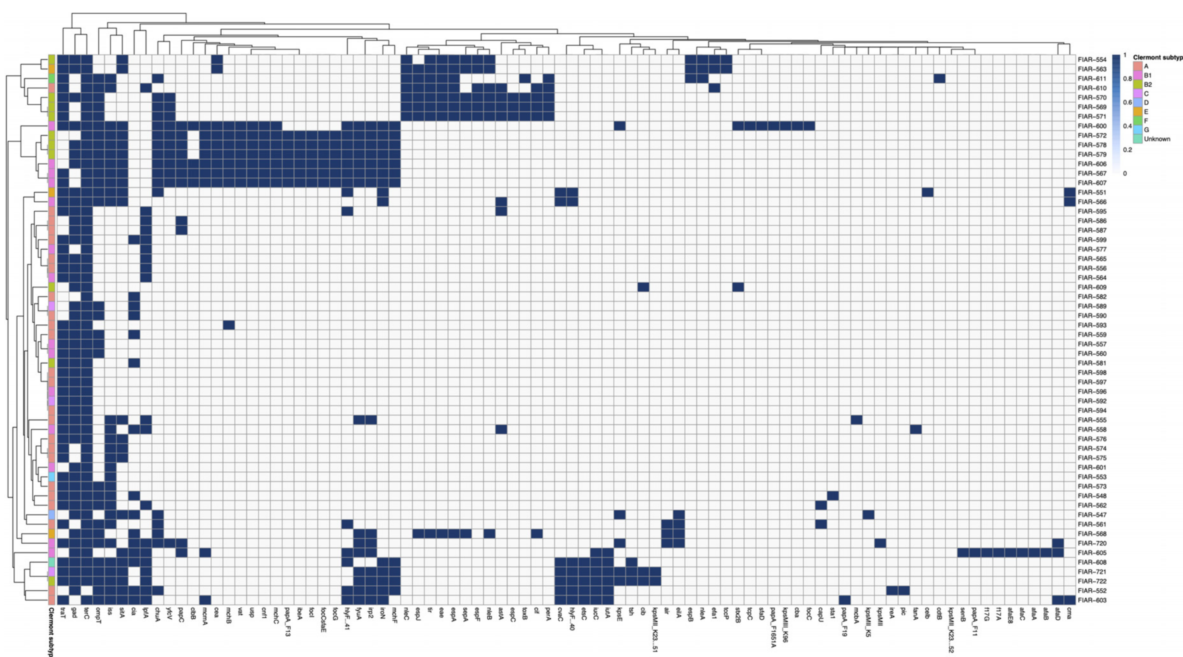

Fig. 3. Hierarchically clustered heatmap of the putative genes distribution of Escherichia coli (n = 58) isolates. The heatmap plot depicts the absence/presence of virulence genes (horizontal axis clustering) within each isolate (variables clustering on the vertical axis). Presence or absence of virulence genes is represented as dark blue or white squares, respectively. The Clermont phylogroups of the isolates are indicated at the left margin of the heatmap.

EilA, was detected in 7% (n = 4) of the isolates. Two isolates harboured Shiga toxin 2 subtype (*stx2b*), with one of them harbouring typical ExPEC-associated genes (Table S4). However, the other *stx2b*-harbouring isolate had a partial alignment, with 100% identity, ending at contig boundaries, which could indicate a split of the gene due to an assembly issue. Regarding the other *stx2b*-harbouring isolate, the identity percentage was 99.6% compared with the complete reference genome. The putative Shiga-toxin–producing *E. coli* (STEC) and ExPEC hybrid belonged to one of the major ExPEC STs, namely ST12, and to phylogroup B2.

3.8. Virulence factors, and polysaccharide capsule and lipopolysaccharide antigen serotype prediction in ESBL-producing K. pneumoniae isolates

Acquired siderophore genes, such as core siderophores (*entA-F*), yersiniabactins (*ybtA, ybtE, ybtP, ybtQ, ybtS, ybtT, ybtU*, and *ybtX*), and salmochelin (*iroE*) genes were detected within the studied *K. pneumoniae* isolates. Moreover, the *K. pneumoniae* ST307 isolate had yersiniabactin locus sequence type 2-2LV, and the yersiniabactin siderophore locus was in the chromosomally integrated ICEKp4 structure variant. The polysaccharide capsule (K) and lipopolysaccharide antigen (O) loci analysis revealed that the *K. pneumoniae* ST307 isolate had the KL102 capsule locus associated with the O2v2 lipopolysaccharide antigen and *wzi* allele 173; the analysis failed to recover any known capsule locus and lipopolysaccharide antigen for the other *K. pneumoniae* isolate with an unknown ST.

3.9. International high-risk clone E. coli ST410

E. coli ST410 was detected in two studied dogs originating from Romania (two isolates, FIAR-586 and FIAR-587) and Hungary (FIAR-605). While the Romanian isolates were found to be both AmpC and ESBL producers harbouring *blaCTX-M-15* and *blaCMY-2*, the Hungarian isolate was classified phenotypically and genotypically as an AmpC producer, harbouring *blaCMY-2*. All these isolates shared similar mutations in QRDRs in their genomes, including S83L and D87N amino acid substitutions in *gyrA*, S80I substitution in *parC*, and S458A substitution in *parE*. Moreover, these isolates harboured *fimH24* and belonged to Clermont subtype C. Only the Hungarian isolate harboured ExPEC-associated virulence factors. The Romanian isolates harboured IncFIA, IncFIB, IncFII, Col-like, and po111 plasmid replicons, with the IncFII belonging to pMLST F1:A-:B49. The Hungarian isolate harboured IncFIA, IncFIB, IncFII, and IncI1-l (gamma) plasmid replicons, with the IncFII belonging to pMLST F26:A4:B1.

3.10. Discussion

We aimed to characterize the genomes of ESBL/AmpC-producing *E. coli* and *K. pneumoniae* isolated from dogs imported or intended to be imported into Finland. Most of the samples (n = 40) were obtained upon the arrival of the dogs, or at the shelters where the dogs were located during the sampling, inferring the local colonization of multidrug-resistant (MDR) bacteria. Our results showed that *E. coli* and *K. pneumoniae* isolates from dogs with a shelter background carried a high diversity of β-lactamase genes, from which the *blaCTX-M-15* gene occurred most frequently. This finding is in agreement with a previous study describing the global prevalence and distribution of β-lactamase genes in ESBL-producing *E. coli* in dogs [2]. Our findings could suggest the introduction of ARGs from numerous sources, but owing to the concurrent occurrence of the *blaCTX-M-15* gene and IncFII replicon in the studied isolates, it is intriguing to hypothesize the possible mediation of such genes through plasmids in shelter settings. Alternatively, the spread of specific clones among sheltered animals could promote the dissemination. However, we recognize the limitations of our study, and concurrent investigations of possible ARG sources, shelter environments, and complete plasmids would be needed to make such conclusions.

We observed globally predominant MDR *E. coli* ST410 and *K. pneumoniae* ST307 clones. While *E. coli* ST410 has been increasingly detected globally in both humans and animals [36], to the best of our knowledge it has not been reported in Finland before. The studied *E. coli* ST410 isolates seem to share the same genomic

Table 1
Prevalence of antibiotic resistance genes in the studied *Escherichia coli* (n = 58) and *Klebsiella pneumoniae* (n = 2) isolates

Antibiotic class and resistance genes	N (%)
Aminoglycoside	
aph(3′)-Ia	4 (6.7)
aph(3′′)-Ib	19 (32.0)
aph(6)-Id	17 (28.0)
aac(6′)-Ib-cr	7 (12.0)
aac(3)-IIa	4 (6.7)
aac(3)-IId	3 (5.0)
aadA1	8 (13.0)
aadA2	7 (12.0)
aadA2b	1 (2.0)
aadA3	1 (2.0)
aadA5	15 (25.0)
aadA16	1 (2.0)
aadA17	1 (2.0)
aadA22	1 (2.0)
aac(3)-IIe	1 (2.0)
Fluoroquinolone	
qnrS1	37 (62.0)
qnrS2	1 (2.0)
qnrB19	2 (3.0)
qnrB6	1 (2.0)
oqxA	4 (6.7)
oqxB	4 (6.7)
Macrolide, lincosamide and stretogramin	
mdf(A)	52 (87.0)
mph(A)	19 (32.0)
erm(B)	1 (2.0)
lnu(F)	5 (8.0)
Phenicol	
cml	1 (2.0)
cmlA1	3 (5.0)
catA1	3 (5.0)
catB3	4 (6.7)
floR	3 (5.0)
Colistin	
mcr-1.1	3 (5.0)
Fosfomycin	
fosA	3 (5.0)
Rifampicin	
ARR-3	2 (3.0)
Sulphonamide	
sul2	19 (32.0)
sul3	4 (6.7)
sul1	17 (28.0)
Tetracycline	
tet(A)	24 (40)
tet(B)	4 (6.7)
dfrA1	1 (2.0)
dfrA8	2 (3.0)
dfrA12	5 (8.0)
dfrA14	7 (12.0)
dfrA17	14 (23.0)
dfrA27	1 (2.0)
dfrA36	1 (2.0)

characteristics with the B3/H24Rx clade, which appears to have evolved by the acquisition of the ESBL-encoding gene *blaCTX-M-15* and an IncFII plasmid [36]. Another MDR high-risk clonal lineage, *K. pneumoniae* ST307, which was detected in one of the studied dogs, has been emerging recently around the globe [37]. In Finland, a specific outbreak of *K. pneumoniae* ST307 was detected in a human patient previously hospitalized in Russia, as well as in an equine hospital [38,39]. In other countries, *K. pneumoniae* ST307 has been detected amongst healthy and sick dogs [40,41]. Like *E. coli* ST410, ESBL-producing *K. pneumoniae* ST307 is often associated with *blaCTX-M-15* and the IncF plasmid [37]. In addition to β-lactamase genes, the studied *K. pneumoniae* ST307 clone harboured genomic features common to the broader *K. pneumoniae* ST307 population [37]. Increasing evidence of reported *E. coli* ST410 and *K. pneumoniae* ST307 in both humans and dogs, some of which belong in the same cluster [36,42], suggests a need for enhanced surveillance and phylogenomic analyses of such clones in the human-animal interface in Finland.

In addition to β-lactamase resistance, the high occurrence of fluoroquinolone resistance in the studied isolates is remarkable. In Finnish canine *E. coli* isolates, enrofloxacin resistance was estimated to be roughly 6% in 2020 [43], while 48 (83%) of the studied *E. coli* isolates and both *K. pneumoniae* isolates were resistant to ciprofloxacin. In contrast to a European-wide study in fluoroquinolone-resistant Enterobacteriaceae, the PMQR determinants played a markedly greater role in mediating fluoroquinolone resistance compared with QRDRs in the studied isolates [44]. This might be due to selective culturing of ESBL-strains that often exhibit co-resistance to fluoroquinolone. The high occurrence of fluoroquinolone-encoding ARGs in the studied samples could be due to conjugative spread of the *blaCTX-M-15* gene by IncF, as reported by previous studies [45]. Indeed, of 51 IncF replicon-carrying isolates, 67% (n = 34) concurrently carried PMQR determinants and the *blaCTX-M-15* gene.

Another interesting finding was the *mcr-1.1*–positive *E. coli* isolated from two dogs originating from the same shelter in Russia. Generally, *mcr* genes are located in bacterial plasmids, with the most frequently reported ones being IncI2, IncX4, and IncHI2 [46], from which IncI2 and IncHI2 were detected in the *mcr-1.1*–positive isolates. Whereas *mcr-1*–positive *E. coli* strains are widely distributed amongst companion animals in Latin America [47] and China [48], fewer studies are available from Europe. In Russia, only sporadic cases of plasmid-mediated *mcr-1* determinants have been reported [49,50]. Because the isolates harbouring *mcr-1.1* genes belonged to different STs, and considering the swift diffusion of *mcr-1* within conjugative elements, it is more likely that the studied shelter dogs have obtained *mcr-1.1* genes through horizontal gene transfer rather than clonal spread of such lineages. In addition, the highest estimated prevalence of *mcr-1* pathogenic *E. coli* is reported to be obtained from food animals (in comparison with humans and food products) [46]; hence, it is possible that the dogs have obtained the *mcr-1.1* gene through feed. As dogs are frequently imported from Russia to Finland, vigilant surveillance of AMR status of imported dogs is recommended.

A considerable proportion of the studied strains qualified in silico as presumptive ExPEC *E. coli*, with the main phylogroup being B2. Phylogroup B2 was detected to be over-represented and positively associated with the presence of numerous virulence factors obtained from clinical UPEC isolates in dogs [51]. Our results show that the studied putative ExPEC isolates belonged to STs associated with urinary tract infections both in humans and dogs. For instance, *E. coli* ST457 has been isolated from patients with urinary tract infection [52]. A recent meta-analysis of global ExPEC lineages revealed that there are other major ExPEC STs (in addition to *E. coli* ST131) in humans, including ST410, ST12, ST127, ST10, and ST117 [53], which were also present in our data. Surprisingly, the studied *E. coli* ST10 strains did not harbour UPEC-specific virulence genes and were therefore considered to be ubiquitous strains. In addition to human-associated UPEC strains, one of the most prevalent STs in our study was ST372, which has been frequently isolated from canine urinary tract infections and is primarily considered a dog-associated strain [51]. The shared genomic similarities between reported human ExPEC lineages and the studied STs could indicate potential exposure of the stray and shelter dogs to human waste.

It is noteworthy that one of the studied putative STEC isolates also carried typical ExPEC-associated virulence genes. While *stx2b* is linked to mild clinical symptoms or asymptomatic faecal carriage [54], these so-called ‘hybrid pathogenic’ strains have recently gained more interest due to their potential to cause systemic infection in combination with gastrointestinal disease [55]. In addi-

tion, with the presence of multidrug resistance, they can be difficult to treat. To the best of our knowledge, there are few data on ExPEC/STEC hybrid strains in companion animals.

Our results highlight the multiplicity of genetic backgrounds disseminating ESBL/AmpC genes, suggesting multiple potential sources of such contamination. In shelter environments, poor hygiene, high animal density, misuse and overuse of antibiotics, and the quality of water and feed may act as contributing factors for the high occurrence of these bacteria. Moreover, as most of the studied shelter dogs originated from the streets, the results could also mirror the overall sanitation level of the country. Further research is warranted on identifying potential drivers responsible for the dissemination of ESBL/AmpC-producing Enterobacteriaceae in animal shelters and amongst stray dogs. The clinical significance of the transmission of these bacteria from imported dogs to other hosts in Finland remains unknown. Because the prevalence of ESBL/AmpC-producing Enterobacteriaceae distinctly exceeds the prevalence of corresponding bacteria in Finnish companion animals and humans, this aspect should be further studied. Lastly, the finding of relevant international clones, as well as *mcr-1*–harbouring ESBL-producing *E. coli*, calls for enhanced antimicrobial resistance surveillance, including molecular characterization of the genomes of companion animals in Finland.

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Competing interests

None declared.

Ethical approval

Not required

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2022.05.021.

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