Community carriage of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae*: a cross-sectional study of risk factors and comparative genomics of carriage and clinical isolates

Niclas Raffelsberger,[1,2] Dorota Julia Buczek,[2] Kristian Svendsen,[3] Lars Småbrekke,[3] Anna Kaarina Pöntinen,[4,5] Iren H. Löhr,[6] Lotte Leonore Eivindsdatter Andreassen,[1] Gunnar Skov Simonsen,[1,2] Norwegian E. coli ESBL Study Group, Arnfinn Sundsfjord,[2,4] Kirsten Gravningen,[1,7,8] Ørjan Samuelsen[3,4]

AUTHOR AFFILIATIONS See affiliation list on p. 13.

ABSTRACT The global prevalence of infections caused by extended-spectrum β-lactamase-producing Enterobacterales (ESBL-E) is increasing, and for *Escherichia coli*, observations indicate that this is partly driven by community-onset cases. The ESBL-E population structure in the community is scarcely described, and data on risk factors for carriage are conflicting. Here, we report the prevalence and population structure of fecal ESBL-producing *E. coli* and *Klebsiella pneumoniae* (ESBL-Ec/Kp) in a general adult population, examine risk factors, and compare carriage isolates with contemporary clinical isolates. Fecal samples obtained from 4,999 participants (54% women) ≥40 years in the seventh survey of the population-based Tromsø Study, Norway (2015, 2016), were screened for ESBL-Ec/Kp. In addition, we included 118 ESBL-Ec clinical isolates from the Norwegian surveillance program in 2014. All isolates were whole-genome sequenced. Risk factors associated with carriage were analyzed using multivariable logistic regression. ESBL-Ec gastrointestinal carriage prevalence was 3.3% [95% confidence interval (CI) 2.8%–3.9%, no sex difference] and 0.08% (0.02%–0.20%) for ESBL-Kp. For ESBL-Ec, travel to Asia was the only independent risk factor (adjusted odds ratio 3.46, 95% CI 2.18–5.49). *E. coli* ST131 was most prevalent in both collections. However, the ST131 proportion was significantly lower in carriage (24%) versus clinical isolates (58%, *P* < 0.001). Carriage isolates were genetically more diverse with a higher proportion of phylogroup A (26%) than clinical isolates (5%, *P* < 0.001), indicating that ESBL gene acquisition occurs in a variety of *E. coli* lineages colonizing the gut. STs commonly related to extraintestinal infections were more frequent in clinical isolates also carrying a higher prevalence of antimicrobial resistance, which could indicate clone-associated pathogenicity.

IMPORTANCE ESBL-Ec and ESBL-Kp are major pathogens in the global burden of antimicrobial resistance. However, there is a gap in knowledge concerning the bacterial population structure of human ESBL-Ec/Kp carriage isolates in the community. We have examined ESBL-Ec/Kp isolates from a population-based study and compared these to contemporary clinical isolates. The large genetic diversity of carriage isolates indicates frequent ESBL gene acquisition, while those causing invasive infections are more clone dependent and associated with a higher prevalence of antibiotic resistance. The knowledge of factors associated with ESBL carriage helps to identify patients at risk to combat the spread of resistant bacteria within the healthcare system. Particularly, previous travel to Asia stands out as a major risk factor for carriage and should be considered in selecting empirical antibiotic treatment in critically ill patients.

Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* (ESBL-Ec) and *Klebsiella pneumoniae* (ESBL-Kp) are major contributors to the global burden of disease due to the antibiotic-resistant bacteria (1). The prevalence of infections caused by ESBL-Ec or ESBL-Kp is increasing, even in countries with low antibiotic use and availability on prescription only, like Norway (2). The mortality of invasive infections with ESBL-producing Enterobacterales (ESBL-E) ranges from 10% to 35%, depending on bacterial species, host factors, severity of disease, and initial antibiotic therapy (3, 4). Consequently, ESBL-Ec/Kp are high-priority pathogens when developing new drugs to combat the threat from antibiotic-resistant bacteria (5).

ESBL-Ec/Kp colonization precedes invasive infections (6–8), and recent reviews report an increasing global prevalence of human ESBL-E carriage in the community, with an eightfold rise over the past two decades (9, 10). The highest prevalence is detected in Asian and African regions (20%–70%) and the lowest in Europe and the Americas (<10%) (9, 10). A study from the USA shows that the increased incidence of ESBL-Ec infections was driven by an increase in community-onset cases (11), and human-to-human transmission was the attributable source to 60% of community-acquired gastrointestinal ESBL-Ec carriage in the Netherlands (12).

Several risk factors for ESBL-E gastrointestinal carriage have been described. However, these have, with a few exceptions (13, 14), mainly been investigated in small and/or selected study populations, such as international travelers (15), patients with gastroenteritis (16), patients recruited by general practitioners (17), persons with recent healthcare contact (18), pregnant women (19), children (20), or persons living in a livestock-dense area (21). Many studies have identified international travel as a risk factor for ESBL acquisition, while the significance of sex, age, antibiotic or proton pump inhibitor use, hospitalization, and diet are conflicting (13–15, 17, 18, 22–24). Factors associated with the carriage of pathogenic bacteria are also shown to overlap. For instance, factors associated with Kp gastrointestinal carriage (25) overlap with those associated with ESBL-Ec carriage (15, 22, 26, 27). Additionally, an association between ESBL-E carriage and vancomycin-resistant enterococci (VRE) has been described previously (7, 28), and also that VRE colonization is significantly associated with Kp colonization among intensive care unit patients (29).

We have comprehensive knowledge of the prevalence and population structure of clinical ESBL-E isolates showing a dominance of CTX-M-group ESBL enzymes and the association with specific extraintestinal pathogenic *E. coli* (ExPEC) and multidrug-resistant *K. pneumoniae* high-risk clones (30–33). Large-scale genomic studies have identified specific subclades of *E. coli* sequence type (ST) 131 and *K. pneumoniae* ST307 as major contributors to the increasing prevalence of ESBL infections (30, 32, 34, 35). Community-based studies from the Netherlands and Sweden also observed a predominance of ST131, but a high genetic diversity within the ESBL-Ec population (13, 14).

Improved knowledge of risk factors for ESBL-E carriage and their population structure in the general human population may provide information for risk stratification and targeted infection control measures. In addition, studies show that it is important to consider ESBL-E carriage in critically ill septic patients with respect to the choice of empirical antibiotic treatment since ESBL-Ec/Kp colonization can precede invasive infections and carriage or non-carriage status may guide empirical antibiotic treatment (36–38). The aims of this study were to examine the prevalence of, and risk factors associated with, ESBL-Ec/Kp gastrointestinal carriage in a general adult population in Norway and to compare the ESBL-Ec population structure with a national collection of clinical isolates.

MATERIALS AND METHODS

Study population and design

Our study sample was drawn from Tromsø7, the last of seven cross-sectional health surveys conducted between 1974 and 2016 in Tromsø municipality, Norway (https://uit.no/research/tromsostudy). Tromsø is representative of a Northern European, urban population (39). Tromsø7 (March 2015–October 2016) included questionnaires and two clinical visits (https://uit.no/research/tromsostudy/project?pid=708909). Unique national identity numbers from the official population registry were used to invite all citizens ≥40 years (n = 32,591). Sixty-five percent (n = 21,083, 11,074 women) attended the first clinical visit in the study (Fig. 1).

A selection of 9,320 persons attending the first visit was invited for a second visit, also including 3,154 former Tromsø Study participants not already included in the random selection process, which was required for other clinical research purposes. From March 2015 to March 2016, 5,800 participants at the first visit were consecutively provided a fecal self-sampling kit. Participants collected fecal material using nylon-flocked ESwab 490CE.A (Copan, Brescia, Italy). In total, 87% (n = 5,042) returned a sample either at the second visit or by mail to the laboratory.

All 5,042 fecal samples were screened for the presence of ESBL-producing Ec and Kp via selective culture (see below). All participants completed two self-administered structured questionnaires on sociodemographics, smoking, alcohol use, hospitalization, drug use, and travel abroad. We excluded 13 participants with the wrong or missing sample identification numbers, two retracting consent to medical research, and 28 with incomplete questionnaires for a final study population of 4,999 participants (Fig. 1). We analyzed the association between ESBL-Ec gastrointestinal carriage and different risk factors in 4,999 participants (Fig. 1). Next, we conducted a sensitivity analysis studying the association between Kp gastrointestinal carriage and ESBL-Ec carriage among 2,973 participants additionally screened for Kp in our previous study (25), irrespective of resistance (Fig. 1).

### Isolation of ESBL-producing *E. coli* and *K. pneumoniae*

We added 200 µL of 85% glycerol to the ESwab tubes on arrival at the local microbiological laboratory and stored the samples at −80°C. From the thawed media, 100 µL were plated onto CHROMagar ESBL (CHROMagar, Paris, France) and incubated for 48 hours at 37°C. Pink, purple, and blue colonies suspected of being ESBL-producing Ec or *Klebsiella* spp. were identified using mass spectrometry (matrix-assisted laser desorption ionization-time of flight [MALDI-TOF]; Bruker Daltonics, Bremen, Germany). The first colony identified as either *E. coli*, *K. pneumoniae*, or *Klebsiella variicola* from each sample was kept and further analyzed. All samples were plated on cysteine lactose electrolyte deficient agar (MAST Group, Bootle, UK) to assess the growth of fecal flora and validity of the samples.

K. pneumoniae isolation

The screening strategy and isolation procedure for Kp gastrointestinal carriage of 2,973 participants in Tromsø7 are described in detail elsewhere (25). Briefly, we plated and screened the fecal samples onto the selective SCAI (Simons citrate agar with inositol; both Sigma-Aldrich, Darmstadt, Germany) medium and identified suspected colonies using MALDI-TOF.

Antimicrobial susceptibility testing and phenotypic ESBL identification

Susceptibility testing was performed according to the European Commitee on Antimicrobial Susceptibility Testing (EUCAST) broth microdilution method for carriage isolates and disc diffusion method (40) for the clinical isolates, and both were interpreted using the EUCAST 2023 clinical breakpoint table (https://eucast.org/). For the confirmation of
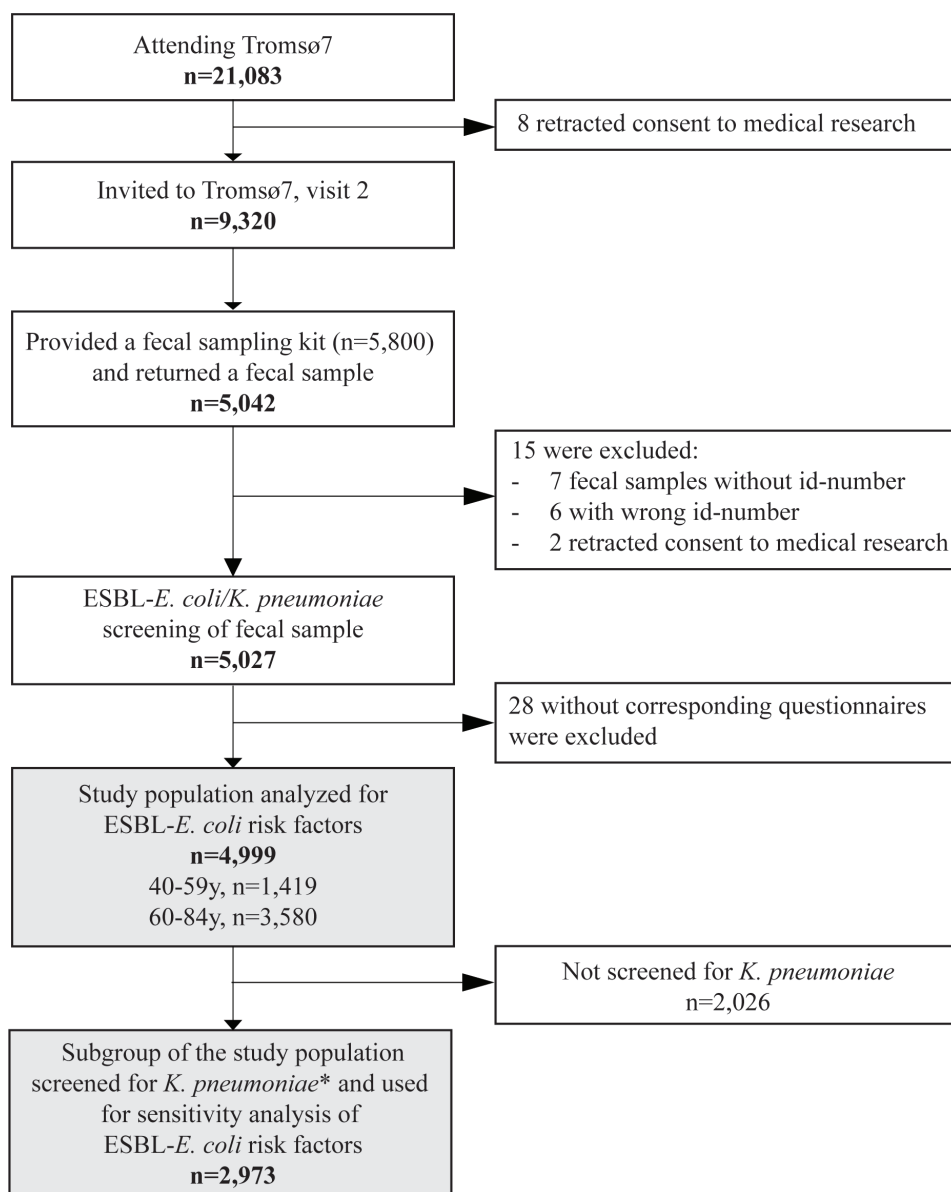
FIG 1 Flow diagram of the study population, Tromsø7, 2015–2016. *Subgroup of the study population screened for *K. pneumoniae* carriage as previously described (25).

ESBL-producing Ec and Kp, we followed the EUCAST algorithm for the phenotypic detection of ESBLs using the BD BBL combination disk test (Becton Dickinson and Company, Sparks, NV, USA).

Genomic sequencing and bioinformatic analysis

Genomic DNA from all 166 ESBL-Ec of the Tromsø7 collection was extracted with the MagNA Pure 96 system (Roche Applied Science, Mannheim, Germany), and sequencing libraries were prepared according to the Nextera Flex sample preparation protocol (Illumina, San Diego, CA, USA). Samples were sequenced on the Illumina MiSeq platform to generate 300-bp paired-end reads. All reads were trimmed with TrimGalore v0.6.4 and assembled with Unicycler v0.4.8 including SPAdes v3.13.0 (41–43). STs were assigned using the multilocus sequence type (MLST) software *E. coli* scheme v2.19.0 and Enterobase (44–46). AMRFinderPlus v3.10.16 was used to determine resistance genes among the Ec isolates (47). Plasmid replicons were identified using Abricate v1.0.1

including the PlasmidFinder 2021-March-27 database (48, 49). Phylogroup assignment was based on ClermonTyping v20.03 (March 2020) (50). The *fimH* type was identified using FimTyper v1.0 and BLAST+ v2.12.0 (51, 52). Regarding the *Klebsiella* isolates, Kleborate v2.0.0 was used to determine species identification, ST, and acquired genes encoding virulence or antibiotic resistance (53, 54).

The clinical ESBL-producing Ec included 118 isolates out of 123 representing all ESBL-Ec isolates collected in 2014 (55), as part of the yearly Norwegian Surveillance Program of Antimicrobial Resistance (NORM). The surveillance program included all clinical microbiological laboratories in Norway. The sampling period was 6 months and 2 days for blood and urine isolates, respectively. Genome sequencing of five isolates was unsuccessful. Before sequencing, the NORM 2014 isolates were stored at −80°C and then sent to GATC Biotech AG (part of Eurofins Genomics/Eurofins Scientific) in Germany for DNA isolation and WGS. Raw reads were trimmed using Trimmomatic v0.39 and assembled with SPAdes v3.15.0 (56, 57). Contigs shorter than 200 bp were discarded. The genomic data were analyzed as described above.

Phylogenetic and population structure analysis of ESBL-*E. coli*

We used Prokka v.1.14.6 (58) to annotate genomes and snippy v.4.6.0 (59) to map the sequence reads to the ST131 Ec EC958 chromosome (HG941718.1) (https://www.ncbi.nlm.nih.gov/nuccore/HG941718.1) to create the core genome alignment with sufficient resolution to differentiate between the ST131 C1 and C2 subclades. We used snp-dists v.0.8.2 (https://github.com/tseemann/snp-dists/) to create the single nucleotide polymorphism (SNP) distance matrix from the core genome alignment. We used a 17 SNP cutoff to define the similarity between two genomes and to identify probable ESBL-Ec transmission events among cases (60). To assess the phylogenetic relatedness, we used the core SNP alignment to infer a maximum-likelihood tree using RAxML v.8.2.8 with the GTR + Gamma rate model and 100 rapid bootstraps visualized in iTol (v6.5.2) (61, 62). ST131 subclades were determined based on subclade-specific SNPs and *fimH* alleles, and subclade membership was corrected when assignment based on the SNP profile of sporadic isolates did not fit with the phylogenetic distribution of clades (30, 35, 51, 63).

Statistical analysis

Our primary analysis was a multivariable logistic regression model, with the outcome variable ESBL-Ec gastrointestinal carriage using SPSS v.26.0 (SPSS, Inc., Chicago, IL, USA). We analyzed factors associated with ESBL-Ec gastrointestinal carriage among 4,999 participants (Table 1). Both the primary (Table 1) and the sensitivity analysis (Table S2) were multivariable logistic regression analyses. Explanatory variables were selected with the help of a directed acyclic graph constructed using DAGitty v3.0 (Fig. S3 and S4) (64). All explanatory variables were kept in the fully adjusted models. Multicollinearity between the entered variables was assessed by calculating the variance inflation factor (VIF) and tolerance statistic. Multicollinearity was not a problem with VIF >10 and tolerance statistic <0.2 (65). The strength of the associations was examined by calculating adjusted odds ratios (AORs) with 95% confidence interval (CI). Two-sided *P*-values <0.05 were considered statistically significant. The prevalence of ST131 among carrier and clinical isolates was compared by calculating the OR with 95% CI using logistic regression in SPSS. The comparison of ESBL-Ec and phenotypic resistance proportions were assessed using χ^2 test.

RESULTS

We detected gastrointestinal carriage of putative ESBL-Ec/Kp in 188 of 4,999 randomly selected participants who provided fecal samples in the seventh survey of the population-based Tromsø study (Tromsø7) (39) (Table S1; Fig. 2). Overall, 87% of participants receiving a sampling kit returned a fecal sample. In total, 180 Ec and 9 Kp putative ESBL-positive isolates were isolated from the 188 ESBL screening-positive fecal samples. Both ESBL-Ec and ESBL-Kp were detected in one sample.

TABLE 1 ESBL-producing *E. coli* gastrointestinal carriage and associated factors among 4,999 participants in Tromsø7[d]

Characteristics	% (ESBL-*E. coli*)	n (ESBL-*E. coli*)	N	AOR	95% CI	P-value
Age (years)						0.232
40–49	3.6	22	605	1.00		
50–59	4.1	33	814	1.15	0.66–2.01	
60–69	3.2	68	2,128	0.81	0.49–1.33	
70–84	3.0	43	1,452	0.72	0.42–1.26	
Hospitalization past 12 mo						0.125
No	3.3	142	4,344	1.00		
Yes	4.0	24	593	1.44	0.90–2.28	
Antibiotic use past 14 days[a]						0.269
No	3.3	158	4,832	1.00		
Yes	5.2	8	155	1.56	0.71–3.43	
Acid-suppressive medication past 4 wk						0.949
Not used	3.3	123	3,762	1.00		
≤Weekly	3.2	13	403	1.02	0.57–1.83	
Every week, but not daily	3.5	9	260	1.10	0.55–2.20	
Daily	3.7	11	297	1.20	0.64–2.27	
Travel abroad past 12 mo[b]						<0.001
No	2.5	53	2,145	1.00		
Other regions (excluding Asia)	3.2	70	2,214	1.36	0.93–1.98	
Asia exclusively or Asia + other regions	8.2	38	462	3.46	2.18–5.49	
Traveler's diarrhea past 12 mo[c]						0.970
No	3.3	156	4,724	1.00		
Yes	4.8	8	166	1.02	0.48–2.16	

[a]Have you taken any antibiotics (tablets or oral suspensions, nasal ointments, eye drops, or eye ointment) during the past 14 days?
[b]Traveled outside the Nordic countries >1-week duration in the past 12 months.
[c]For each travel abroad the past 12 months, the participants were asked if they had experienced diarrhea in connection with the travel.
[d]ESBL, extended-spectrum β-lactamase; N, denominator; AOR, adjusted odds ratio; CI, confidence interval. AOR adjusted for age, hospitalization in the past 12 months, antibiotic use in the past 14 days, acid suppressive medication in the past 4 weeks, travel abroad in the past 12 months, and traveler's diarrhea in the past 12 months.The multivariable model includes 4,491 participants with complete information on all variables.

Phenotypic and genotypic analyses showed that 14 putative ESBL-Ec isolates were either phenotypically and/or genotypically ESBL-negative. Of these, one isolate harbored plasmid-mediated AmpC (bla_{CMY-2}). After exclusion of the ESBL-negative isolates, the prevalence of ESBL-Ec gastrointestinal carriage was 3.3% (95% CI 2.8%–3.9%, 166 of 4,999 participants): 3.1% (2.5%–3.9%) in women and 3.5% (2.8%–4.4%) in men.

Among the nine screening ESBL-positive Kp isolates, one harbored bla_{CMY-2} and four did not express an ESBL phenotype. Consequently, four samples were considered positive for ESBL-producing Kp, corresponding to a prevalence of 0.08% (0.02%–0.20%), all among male participants.

Factors associated with gastrointestinal carriage of ESBL-*E. coli*

We analyzed data from 4,999 participants (Table S1). Median age was 65 years (interquartile range 58–70 years, no sex difference) of the whole study population. In multivariable logistic regression analyses adjusted for all explanatory variables, only travel to Asia in the past 12 months was associated with ESBL-Ec gastrointestinal carriage with an AOR of 3.46 (2.18–5.49) (Table 1). Among participants reporting hospitalization in the past year, or recent use of antibiotics or acid-suppressive medication, we observed a non-significant increase in the prevalence of ESBL-Ec.

Considering the overlapping risk factors between ESBL-E and Kp gastrointestinal carriage identified in the literature (15, 22, 26, 27) and in our previous study (25), we conducted a sensitivity analysis with Kp carriage as a risk factor in a subgroup of 2,973 participants (of the total 4,999 participants in this current study) previously screened for Kp (Table S2). In this model including Kp carriage, AOR was 1.65 (0.98–2.77), indicating a possible association between Kp and ESBL-Ec carriage (Table S2). However, no significant
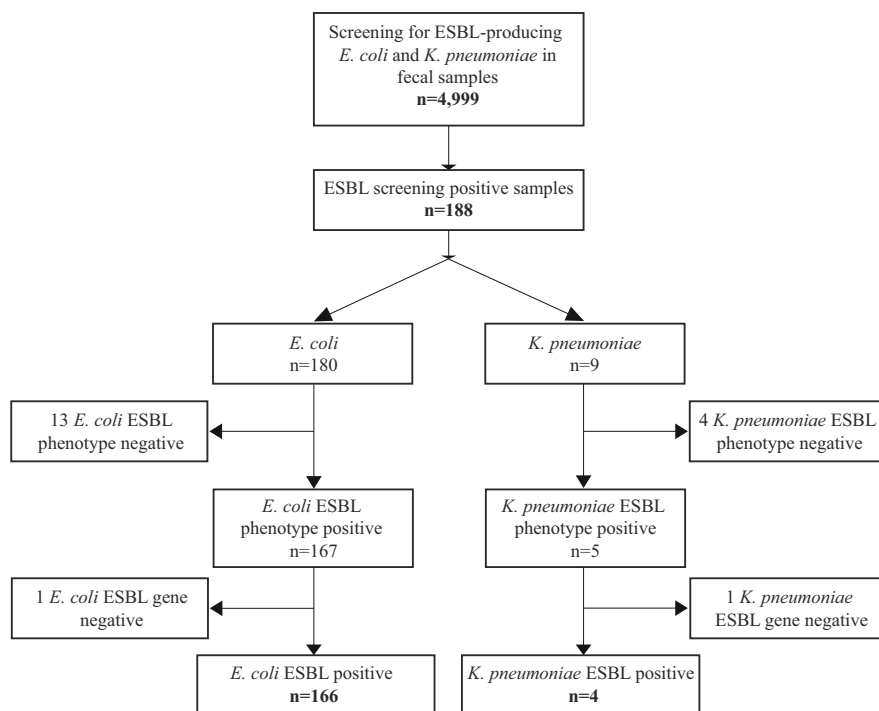
FIG 2 Flowchart and results of fecal sample screening for gastrointestinal carriage of ESBL-producing *E. coli* and *K. pneumoniae* in 4,999 participants in Tromsø7, 2015–2016.

differences in the estimates of the risk factors were observed compared to the model without Kp carriage conducted on the whole study population (Table 1; Table S2), indicating the validity of our primary model.

Comparative analysis of ESBL-*E. coli* carriage and clinical isolates

To explore the population structure and genomic characteristics of ESBL-Ec in community carriage, we whole-genome sequenced (WGS) all 166 isolates. Furthermore, we sequenced a contemporary national collection of 118 clinical ESBL-Ec isolates (NORM 2014) for comparative analysis (Fig. 3; Table S3).

At the phylogroup level, 52.4% of the ESBL-Ec carriage isolates belonged to either phylogroup A (26.5%) or D (25.9%), and 33.7% belonged to phylogroup B2 (Fig. 3 and 4; Table S3). This contrasts with the clinical isolates where 64.4% ($P < 0.001$) of the isolates belonged to phylogroup B2 and only 20.4% ($P < 0.001$) belonged to phylogroup A (5.1%) and D (15.3%) combined.

Carriage isolates had higher ST diversity (Fig. 3 and 4; Table S3) with Simpson's diversity index of 92.4% compared to clinical isolates (65.9%, $P < 0.001$). We identified 58 different STs among the carriage isolates while the clinical isolates included 27 STs (Fig. 3 and 4; Table S3). ST131 (phylogroup B2) was the dominant ST in both collections, however, more prevalent among clinical isolates (57.6%, $n = 68$) than carriage isolates (24.1%, $n = 40$, $P < 0.001$). Comparison of the prevalence of ESBL-producing ST131 colonization versus infection in our study results in a crude odds ratio (OR) for infection of 4.3 (2.57–7.13, $P < 0.001$).

Within ST131, the multidrug-resistant subclades C1 (alternatively referred to as *H*30-R) and C2 (*H*30-Rx) accounted for 67.5% in carriage and 73.5% in clinical isolates (Fig. 3; Fig. S1 and Table S3). The subclade C1 (47.5%, $n = 19$) was the most prevalent among carriage isolates and C2 (42.6%, $n = 29$) among clinical isolates. The difference in the proportion of C2 between clinical and carriage isolates (20.0%, $n = 8$, $P = 0.017$) was statistically significant. For the overall ESBL-Ec population, crude OR for infection among C2 was 6.44 (2.82–14.68, $P < 0.001$) (Table S4).
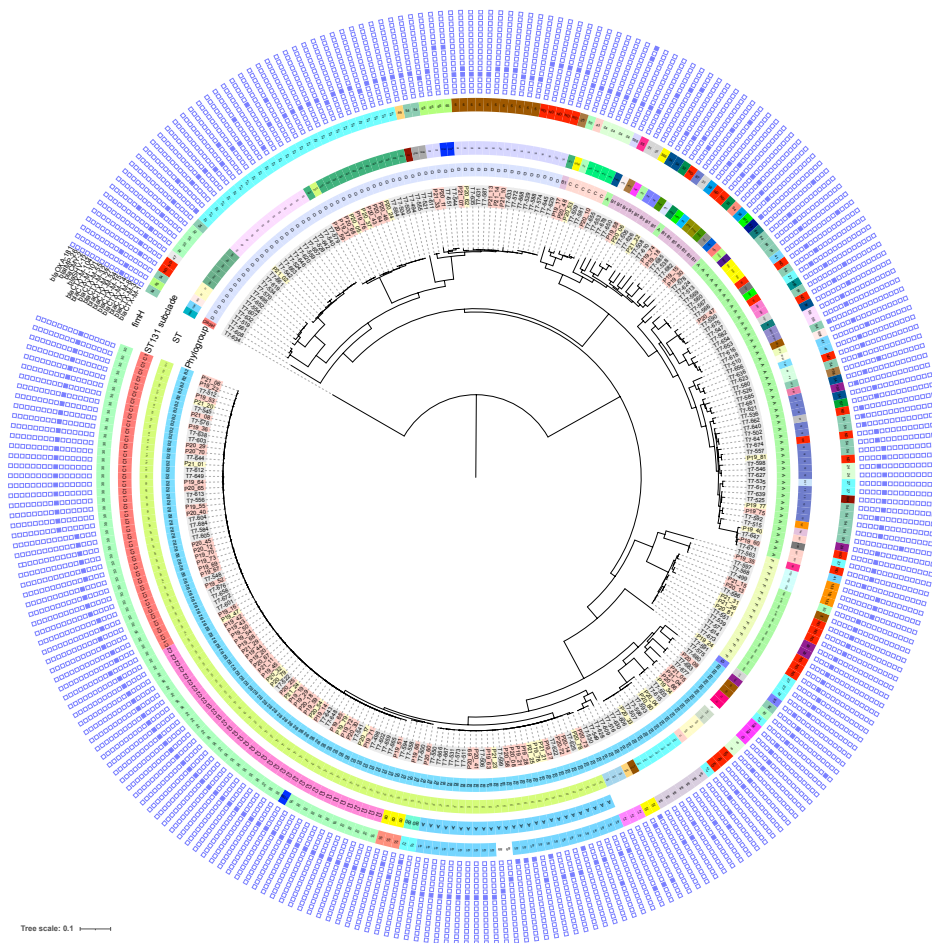
FIG 3 Maximum-likelihood phylogenetic tree based on core genome alignment of the genomes of ESBL-*E. coli* carriage isolates from Tromsø7 (labeled gray, *n* = 166) and clinical isolates from NORM 2014 (blood isolates labeled with red and urine isolates with yellow, *n* = 118). The innermost ring illustrates phylogroups, followed by a ring with sequence types (STs), a ring with ST131 subclades, and a ring with *fimH* types (ND, not detected). The heatmap shows the presence (blue color) or absence (white) of ESBL gene variants and other relevant β-lactamases.

The proportion of subclades A, B, and B0, less associated with antibiotic resistance, was not different between ST131 carriage isolates (32.5%) and clinical isolates (26.5%, *P* = 0.508) (Fig. S1). However, with regard to the overall ESBL-Ec population, subclade A was associated with infection (carriage 6.0% versus clinical 13.6%, OR 2.45, 1.07–5.60, *P* = 0.034) (Table S4).

The most prevalent STs in carriage isolates following ST131 were ST10 (7.2%, *n* = 12, phylogroup A), ST69 (7.2%, *n* = 12), and ST38 (6.6%, *n* = 11, both phylogroup D) compared to ST405 (7.6%, *n* = 9), ST38 (5.9%, *n* = 7, both phylogroup D), and ST648 (5.1%, *n* = 6, phylogroup F) in clinical isolates (Fig. 3 and 4; Table S3). We did not identify the common ExPEC lineage ST73 (30, 34) among the carriage isolates but found one ST95 (30, 34) (0.6%) and five isolates of the emerging ST1193 (66, 67) (3.0%).

Using an SNP cutoff of ≤17 (60), we detected 2 putative clusters (Table S5) among 5 of 284 isolates. All five were carriage isolates. One ST357 cluster (6–9 SNP differences) consisted of three isolates and the other cluster of two ST131 isolates (4 SNP differences). We detected no clusters among the clinical isolates.

Antimicrobial resistance and plasmid replicon content

CTX-M enzymes accounted for at least 97.0% of the ESBL phenotypes in both collections (Fig. 3 and 5; Table S3). The most prevalent ESBL genes among both carriage and clinical
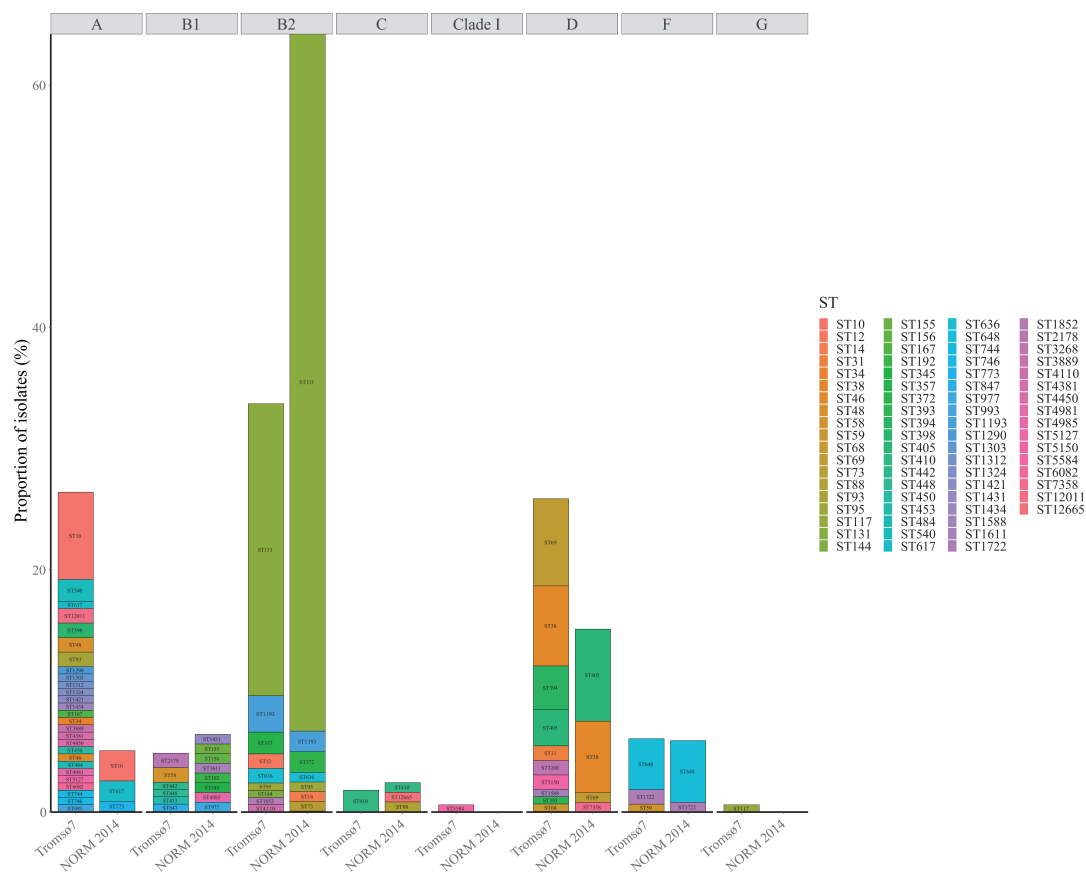
FIG 4 Phylogroup (labeled on the top) and sequence type (ST) distribution of ESBL-*E. coli* carriage isolates from Tromsø7 (*n* = 166) and clinical isolates from NORM 2014 (*n* = 118).

isolates were $bla_{CTX-M-15}$ (40.4%, *n* = 67 versus 61.0%, *n* = 72, *P* = 0.001), $bla_{CTX-M-14}$ (20.5%, *n* = 34 versus 14.4%, *n* = 17, *P* = 0.188), and $bla_{CTX-M-27}$ (19.9%, *n* = 33 versus 15.3%, *n* = 18, *P* = 0.321). One clinical isolate of ST38 harbored both $bla_{CTX-M-15}$ and $bla_{CTX-M-14}$. $bla_{CTX-M-3}$, $bla_{CTX-M-8}$, $bla_{CTX-M-32}$, and $bla_{CTX-M-101}$ were exclusively detected in carriage isolates, whereas $bla_{CTX-M-24}$ and $bla_{CTX-M-104}$ were present only in clinical isolates. The remaining ESBL producers contained bla_{SHV-12} in 3.0% of the carriage and in 1.7% of the clinical isolates. bla_{TEM} ESBL genes were not detected.

The clinical isolates showed an overall higher proportion of phenotypic resistance compared to the carriage isolates (Table 2; Table S3). Only 2.4% of carriage isolates were phenotypically resistant to piperacillin–tazobactam compared to 31.8% (*P* < 0.001) of clinical isolates. We also detected a high prevalence of phenotypic co-resistance to non-β-lactam antibiotics, such as gentamicin, ciprofloxacin, and trimethoprim–sulfamethoxazole, both in clinical and carriage isolates. Isolates co-resistant to all these three antibiotic classes accounted for 10.2% (17/166) of carriage and 33.1% (39/118, *P* < 0.001) of clinical isolates.

Phenotypic ciprofloxacin resistance was different for ST131 (70.0%) versus non-ST131 (26.2%, *P* < 0.001) among carriage isolates in contrast to the clinical isolates (Table S6). Two carriage isolates of ST484 and ST1324 (both phylogroup A) were phenotypically resistant to colistin and harbored *mcr*-1.1 and *mcr*-3.5, respectively. No isolates expressed clinical resistance against carbapenems. However, two clinical isolates of ST95 (phylogroup B2) and ST410 (phylogroup C) harbored bla_{IMP-26} and $bla_{OXA-181}$, respectively. All isolates were susceptible to tigecycline.

Despite the overall higher proportion of phenotypic resistance among clinical isolates, the average number of different plasmid replicon-types per isolate (both 3.1/ isolate) did not differ between the carriage and clinical collections. We identified 43 and

TABLE 2 Susceptibility profile of ESBL-*E. coli* carriage isolates from Tromsø7 (*n* = 166) and clinical isolates from NORM 2014 (*n* = 118)[d]

	Carriage			Clinical		
	%S	%I	%R	%S	%I	%R
Amoxicillin–clavulanic acid i.v.	57.2	–	42.8	25.4	–	74.6
Amoxicillin–clavulanic acid[a]	90.4	–	9.6	54.2	–	45.8
Piperacillin–tazobactam	97.6	–	2.4	68.2[b]	–	31.8[b]
Cefuroxime[a]	0.6	–	99.4	1.7	–	98.3
Ceftazidime	20.5	32.5	47.0	10.2	11.0	78.8
Cefotaxime	0.0	2.4	97.6	0.8	0.8	98.4
Cefepime	7.8	54.8	37.3	4.7[b]	5.9[b]	89.4[b]
Ceftazidime–avibactam	100.0	–	0.0	NA	NA	NA
Ertapenem	100.0	–	0.0	NA	NA	NA
Meropenem	100.0	0.0	0.0	99.2	0.8	0.0
Aztreonam	5.4	23.5	71.1	NA	NA	NA
Amikacin	100.0	–	0.0	NA	NA	NA
Gentamicin	80.1	–	19.9	48.3	–	51.7
Tobramycin	76.5	–	23.5	NA	NA	NA
Ciprofloxacin	49.4	13.9	36.7	14.4	8.5	77.1
Trimethoprim–sulfamethoxazole	48.8	0.6	50.6	27.1	0.0	72.9
Nitrofurantoin	99.4	–	0.6	93.9[c]	–	6.1[c]
Fosfomycin	99.4	–	0.6	NA	NA	NA
Fosfomycin[a]	96.4	–	3.6	NA	NA	NA
Colistin	98.8	–	1.2	NA	NA	NA
Tigecycline	100.0	–	0.0	100.0[b]	–	0.0[b]

[a]Breakpoints for uncomplicated urinary tract infections.
[b]Available for blood culture isolates only (*n* = 85).
[c]Available for urine culture isolates only (*n* = 33).
[d]S, susceptible; I, susceptible, increased exposure; R, resistant; i.v., intravenous; –, no I category; NA, not available.

39 different plasmid replicon types among 97.1% (162/166) carriage and 95.0% (112/118) clinical isolates (Table S3; Fig. S2). The most prevalent were IncFIB(AP001918) (carriage 22.7% versus clinical 22.3%) followed by Col156 (carriage 11.1% versus clinical 11.5%) and IncFIA (carriage 10.9% versus clinical 17.7%, *P* = 0.101). Replicon type Col156 and IncFIA were mainly detected in globally disseminated ExPEC clones (ST131, ST1193, ST648, ST69, ST405), whereas IncFIB(AP001918) was additionally frequently spread among other STs (Table S3).

ESBL-*K. pneumoniae* carriage isolates

We detected only four ESBL-Kp, each of a different ST (ST29, ST211, ST261, and ST2459) harboring $bla_{CTX-M-15}$ (*n* = 2), $bla_{CTX-M-14}$ (*n* = 1), or bla_{SHV-12} (*n* = 1). None of the isolates were genomically assigned with a virulence score >1 using Kleborate (53).

DISCUSSION

Our study contributes to the knowledge of prevalence of, and factors associated with, ESBL-Ec/Kp gastrointestinal carriage in a general adult population and the bacterial population structure of carriage isolates. The comparison to a contemporary collection of clinical ESBL-Ec isolates revealed differences in the population structure and the prevalence of phenotypic resistance between carriage and clinical isolates. Travel to Asia was identified as a major risk for ESBL-Ec gastrointestinal carriage.

An ESBL-Ec carriage prevalence of 3.3% (2.8%–3.9%) is lower but comparable to previous community-based data from Europe including Sweden 4.4% (3.5%–5.3%, *n* = 2,134; data collected 2012–2013) (13), the Netherlands 4.5% (3.9%–5.1%, *n* = 4,177; 2014–2016) (14), and a Norwegian study 4.9% (2.7%–8.1%, *n* = 284; 2014–2016) (17) using similar screening approaches.
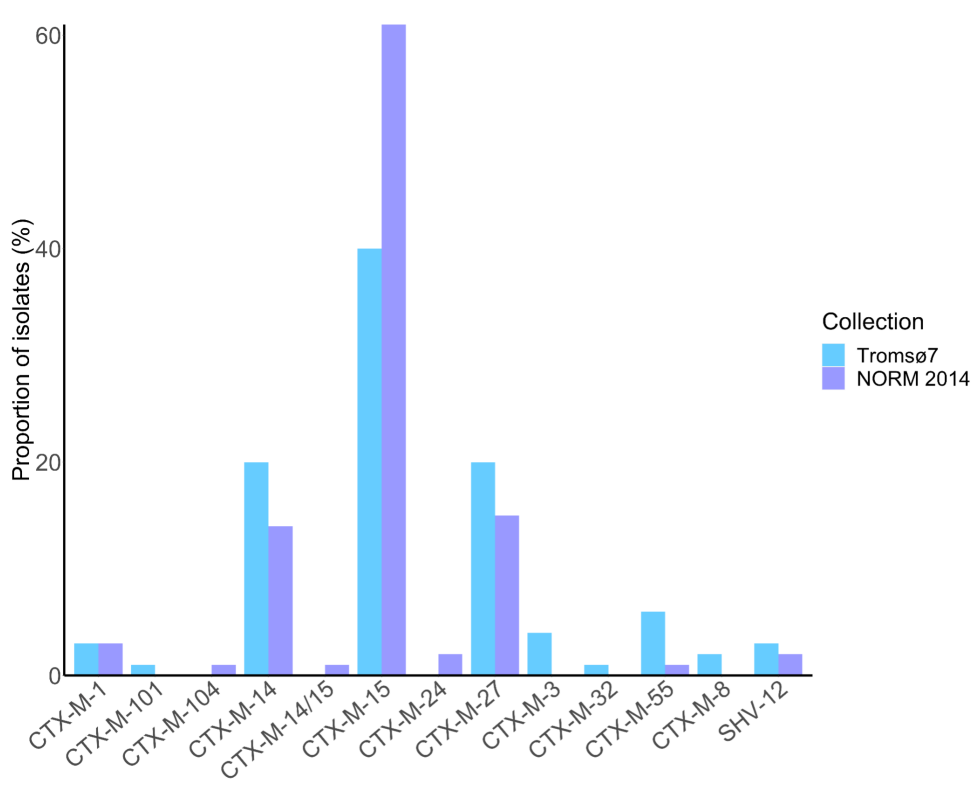
FIG 5 ESBL gene prevalence in ESBL-*E. coli* carriage isolates from the Tromsø7 study (*n* = 166) and clinical isolates from NORM 2014 (*n* = 118).

We identified significant differences in the ESBL-Ec population structure between the community and clinical isolates. The globally disseminated phylogroup B2 clone ST131 has been identified as a key contributor to the increase in ESBL prevalence (68) and in a longitudinal study of *E. coli* bloodstream isolates we identified ST131 to be the single largest contributor to the increase in the prevalence of ESBL-Ec in Norway (30). We also observed the predominance of ST131 in both of our collections. However, the proportion is significantly lower in carriage isolates due to lower numbers of the multidrug-resistant subclade C2. Moreover, the carriage population had a higher proportion of phylogroup A, associated with asymptomatic intestinal carriage in humans (69, 70), and a significantly greater ST diversity overall, compared to the clinical isolates. These observations indicate that the acquisition of ESBL genes frequently occurs in a variety of *E. coli* lineages colonizing the gut. However, there are differences in the colonization potential of *E. coli* lineages and the risk of invasive infection by ESBL-Ec which seems to be clone dependent (68, 71). The higher odds for the infection that we detected for ST131 is similar to that of the Swedish study (AOR 3.4, 1.8–6.4) indicating a higher pathogenicity potential of ST131 compared to commensal *E. coli* lineages of phylogroup A, such as ST10 (13). Moreover, we found that ST131 subclade A, previously reported with less resistance, and the multidrug-resistant subclade C2 had higher odds for infection, and this may contribute to the sustained establishment of these subclades among bloodstream infections in Norway (30).

Assuming a 100% colonization rate of *E. coli*, the large proportion of STs notorious as common causes of extraintestinal clinical infections (e.g., ST131, ST405, ST38, and ST648) (30, 32, 33) could at least partly explain the higher prevalence of ESBL among *E. coli* causing bloodstream infections in Norway (5.8% in 2016) (72) compared to the carriage prevalence of 3.3% identified here. We also observed emerging clones such as ST1193, which appears to have disseminated rapidly worldwide over the last decade (66, 67). The

low prevalence of ESBL-Kp gastrointestinal carriage (0.08%) is consistent with previous community-based reports (9, 14, 17).

The identification of clusters with closely related isolates could indicate putative clonal spread. This could include within-household and social network transmission or nosocomial spread. However, we did not have access to epidemiological data to examine this further.

In line with other studies, we found a strong association between ESBL-Ec carriage and travel to Asian regions (13–15, 17, 22). This supports the current patient screening recommendations for ESBL-producing Gram-negative bacteria after a hospital stay abroad in the past year before hospital admission in Norway (73). In contrast to a Swedish and a Dutch study (15, 74), we did not identify travelers' diarrhea as a risk factor for ESBL-Ec carriage. However, our study was not designed to specifically investigate international travelers but rather focused on risk factors in the general adult population.

We found no association between ESBL-Ec gut carriage and factors such as hospitalization, antibiotic use, and acid-suppressive medication, and conflicting results have been detected in previous studies (22, 75). Hospitalization as a risk factor has mainly been reported in studies investigating patients with ESBL-E infections (23, 76). In line with most studies that assessed risk factors regarding ESBL-E carriage in individuals in the community, we did not identify hospitalization as an independent risk factor (13, 22, 74). This may be due to the increased ESBL prevalence not only in hospitals but also in the community over the last decades and implies that boundaries have become blurred between those two settings (9, 22, 77, 78).

The non-significant effect of antibiotic use is mainly due to the limitations of the drug variable in our study which is based on self-reported data, including topical antibiotics and covers only the last 2 weeks before self-sampling. As antibiotic use has been found as a risk factor for resistance in many other studies (14, 15, 22), we cannot rule out that antibiotic use plays a role in ESBL-E carriage. There are reports identifying an association between the use of gastric acid–suppressive medication and intestinal colonization or infections with ESBL-E (18, 21, 23, 26, 27, 76). However, a Dutch study comparable to ours did not find an association between proton pump inhibitor use and ESBL-E carriage in the overall analysis (14).

Interestingly, we found a possible association between Kp and ESBL-Ec carriage. This may support the previously described link between ESBL-E, VRE (7, 28), and Kp colonization among intensive care unit patients (29). These associations warrant further investigations to assess if a common set of risk factors for carriage of different clinically important pathogens can be identified.

Decolonization of ESBL-E gastrointestinal carriage has been investigated as a possible strategy to reduce the risk of infection and transmission. However, decolonization is not recommended due to insufficient evidence (79). Our findings could also be considered in designing future decolonization strategies.

An important strength of our study is the non-selective recruitment from the official population registry, and the high participation (87%) compared to 18.3% and 18.8% in comprehensive studies from the Netherlands (14) and Sweden (13), respectively. It is a limitation that we only captured the general population 40 years and older. However, other studies have not found an association between age and ESBL-E carriage (13, 14). If age is associated with specific Ec STs, this might potentially bias the ST-specific estimates. Moreover, more extensive data on drug use would have strengthened the analyses. Additionally, the genomic diversity of carriage isolates is likely to be underestimated due to the isolation and sequencing of only one colony per fecal sample. A previous nationwide genomic study on *E. coli* causing bloodstream infections detected no discernible spatiotemporal spread or phylogenetic structure within Norway (30), indicating limited bias in comparing a local and national collection of isolates. The short-read data did not allow us to investigate differences in plasmid population structure between carriage and clinical isolates beyond the level of replicon types.

Conclusions

The prevalence of ESBL-Ec carriage in a general adult urban Norwegian population was low reflecting the relatively low prevalence of ESBL-Ec in clinical isolates. Travel to Asia was the only independent risk factor for ESBL-Ec carriage and should be considered in terms of screening recommendations before hospital admission. The differences in ESBL-Ec populations between carriage and clinical isolates indicating a higher risk of infection dependent on the ESBL-Ec clone support the integration of genomics in risk assessments.

AUTHOR AFFILIATIONS

[1]Department of Microbiology and Infection Control, University Hospital of North Norway, Tromsø, Norway
[2]Department of Medical Biology, Faculty of Health Sciences, UiT The Arctic University of Norway, Tromsø, Norway
[3]Department of Pharmacy, Faculty of Health Sciences, UiT The Arctic University of Norway, Tromsø, Norway
[4]Norwegian National Advisory Unit on Detection of Antimicrobial Resistance, Department of Microbiology and Infection Control, University Hospital of North Norway, Tromsø, Norway
[5]Department of Biostatistics, University of Oslo, Oslo, Norway
[6]Department of Medical Microbiology, Stavanger University Hospital, Stavanger, Norway

⁷Department of Microbiology and Infection Control, Akershus University Hospital, Nordbyhagen, Norway

⁸Division of Medicine and Laboratory Sciences, Institute of Clinical Medicine, University of Oslo, Oslo, Norway

AUTHOR ORCIDs

Niclas Raffelsberger http://orcid.org/0000-0002-9463-8915
Dorota Julia Buczek http://orcid.org/0000-0002-2282-6298
Kristian Svendsen http://orcid.org/0000-0003-3481-3539
Lars Småbrekke http://orcid.org/0000-0001-6475-3368
Anna Kaarina Pöntinen http://orcid.org/0000-0002-3160-2042
Iren H. Löhr http://orcid.org/0000-0002-8847-0044
Gunnar Skov Simonsen http://orcid.org/0000-0003-0043-7045
Arnfinn Sundsfjord http://orcid.org/0000-0002-3728-2270
Kirsten Gravningen http://orcid.org/0000-0002-8194-3208
Ørjan Samuelsen http://orcid.org/0000-0002-5525-2614

AUTHOR CONTRIBUTIONS

Niclas Raffelsberger, Conceptualization, Data curation, Formal analysis, Investigation, Validation, Visualization, Writing – original draft, Writing – review and editing | Dorota Julia Buczek, Data curation, Formal analysis, Investigation, Visualization, Writing – review and editing | Kristian Svendsen, Conceptualization, Data curation, Formal analysis, Investigation, Supervision, Validation, Visualization, Writing – review and editing | Lars Småbrekke, Conceptualization, Data curation, Formal analysis, Investigation, Supervision, Validation, Visualization, Writing – review and editing | Anna Kaarina Pöntinen, Data curation, Formal analysis, Investigation, Visualization, Writing – review and editing | Iren H. Löhr, Conceptualization, Funding acquisition, Resources, Supervision, Writing – review and editing | Lotte Leonore Eivindsdatter Andreassen, Data curation, Formal analysis, Investigation, Writing – review and editing | Gunnar Skov Simonsen, Data curation, Resources, Writing – review and editing | Norwegian *E. coli* ESBL Study Group, Data curation, Resources, Writing – review and editing | Arnfinn Sundsfjord, Conceptualization, Funding acquisition, Resources, Supervision, Writing – review and editing | Kirsten Gravningen, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Supervision, Validation, Writing – original draft, Writing – review and editing | Ørjan Samuelsen, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing

DATA AVAILABILITY STATEMENT

Bacterial genome data (raw Illumina reads) are publicly available in National Center for Biotechnology Information under BioProject PRJEB53319 (NORM 2014 collection, https://www.ebi.ac.uk/ena/browser/view/PRJEB53319) and PRJEB57251 (Tromsø7

collection, https://www.ebi.ac.uk/ena/browser/view/PRJEB57251). This study is based on data owned by a third party (The Tromsø Study, Department of Community Medicine, UiT The Arctic University of Norway, Norway). Confidentiality requirements according to the Norwegian law prevent sharing of individual patient level data in public repositories. Application of legal basis and exemption from professional secrecy requirements for the use of personal health data in research may be sent to a regional committee for medical and health research ethics (https://rekportalen.no/). The authors gained access to the data through the Tromsø Study's application process. Guidelines on how to access the data are available at the website https://uit.no/research/tromsostudy. All inquiries about the Tromsø Study should be sent by e-mail to tromsous@ism.uit.no. All the questionnaire variables are published in the NESSTAR program system, and results can be viewed online: http://tromsoundersokelsen.uit.no/tromso/.

ETHICS APPROVAL

The study was approved by the Regional Committee for Medical and Health Research Ethics, North Norway (REC North reference: 2016/1788 and 2014/940) and the Data Protection Officer at University Hospital of North Norway (reference: 2019/4264). The study complied with the Declaration of Helsinki. All participants in Tromsø7 signed an informed consent form prior to participation.

ADDITIONAL FILES

The following material is available online.

Supplemental Material

Fig. S1 (mSphere00025-23-s0001.pdf). ST131 clade distribution among ESBL-*E. coli* carriage isolates from Tromsø7 (*n*=40) and clinical isolates from NORM 2014 (*n*=68).

Fig. S2 (mSphere00025-23-s0002.pdf). Replicon type distribution among ESBL-*E. coli* from carriage isolates from Tromsø7 (*n*=166) and clinical isolates from NORM 2014 (n=118).

Fig. S3 (mSphere00025-23-s0003.pdf). Directed acyclic graph (DAG) illustrating the causal relationship between ESBL-*E. coli* (ESBL-Ec) gastrointestinal carriage (outcome) and relevant covariates among 4,999 participants in Tromsø7. The variable 'drug use' includes antibiotic use past 14 days and acid suppressive medication past four weeks. To set up the most plausible causal relationship between covariates and the outcome, we first searched the literature for relevant factors associated with ESBL-*E. coli* gastrointestinal carriage. Thereafter, the DAG guided the selection of the multivariable logistic regression model, which was adjusted for the minimal sufficient adjustment set comprising age, drug use, hospitalization, travel abroad, and traveler`s diarrhea (i.e. the variables constituting a confounding pathway). Controlling for the minimal sufficient adjustment set warrants that confounding paths are blocked in order to minimize bias of the causal relationship. The model was not adjusted for sex due to no statistically significant sex difference in prevalence of ESBL-*E. coli* carriage and because it does not constitute biasing paths after adjustment. For the variables sex and alcohol consumption, a direct effect on ESBL-*E. coli* carriage is not described or investigated, however they are known to affect the microbiota composition. Hence, intestinal microbiota was included as an unobserved mediator of the causal relationship.

Fig. S4 (mSphere00025-23-s0004.pdf). Directed acyclic graph (DAG) illustrating the causal relationship between *K. pneumoniae* species complex (Kp) gastrointestinal carriage (exposure), ESBL-*E. coli* (ESBL-Ec) gastrointestinal carriage (outcome), and relevant covariates among 2,973 participants in Tromsø7. The variable 'drug use' includes antibiotic use past 14 days and acid suppressive medication past four weeks. *K. pneumoniae* is a common cause of healthcare associated infections often combined with antimicrobial resistance. In the literature and our previous study (Raffelsberger N, Hetland MAK, Svendsen K, et al. Gastrointestinal carriage of *Klebsiella pneumoniae*

in a general adult population: a cross-sectional study of risk factors and bacterial genomic diversity. Gut Microbes. 2021 Jan-Dec;13(1):1939599.), we identified several factors associated with *K. pneumoniae* gastrointestinal carriage, which are overlapping with those associated with ESBL-*E. coli* carriage. To capture the relevance of *K. pneumoniae* carriage as an exposure, we included this variable into the causal relationship with ESBL-*E. coli* carriage. The DAG was used for the selection of variables for the multivariable logistic regression model, to conceptualize confounding and to identify the minimal sufficient adjustment set. Although sex and alcohol consumption represent ancestors of exposure and outcome, the adjustment for age, drug use, hospitalization, travel abroad, and traveler`s diarrhea controls for relevant confounders and blocks biasing paths. The absence of red arrows in the DAG implies that there are no open unadjusted confounding pathways.

Table S1 (mSphere00025-23-s0005.pdf). Characteristics of the study population.

Table S2 (mSphere00025-23-s0006.pdf). Sensitivity analysis.

Table S3 (mSphere00025-23-s0007.csv). Genome characteristics of ESBL-*E. coli* from carriage isolates from Tromsø7 (n=166) and clinical isolates from NORM 2014 (*n*=118) isolates.

Table S4 (mSphere00025-23-s0008.pdf). Comparison of ESBL-*E. coli* ST131 subclade prevalence.

Table S5 (mSphere00025-23-s0009.pdf). SNP distances among two putative ESBL-producing *E. coli* clusters.

Table S6 (mSphere00025-23-s0010.pdf). Susceptibility profile of carriage and clinical isolates of ESBL-*E. coli* ST131 and non-ST131.

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