Epidemiology of Extended Spectrum β-lactamase-producing *E. coli* and vancomycin-resistant enterococci in the Northern Dutch-German cross- border region.

3

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Running title: Epidemiology of ESBL and VRE in hospitals and the community

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ABSTRACT

Objectives; To reveal the prevalence and epidemiology of Extended spectrum β-lactamase (ESBL)- and/or plasmid AmpC (pAmpC)- and carbapenemase (CP) producing *Enterobacteriaceae* and vancomycin resistant enterococci (VRE) across the Northern Dutch-German border region.

Methods; A point-prevalence study on ESBL/pAmpC,/CP producing *Enterobacteriaceae* and VRE was carried out in hospitalized patients in the Northern Netherlands (n=445, 2012-2013) and Germany (n=242, 2012). Healthy individuals from the Dutch community (n=400, 2010-2012) were also screened. In addition, a genome-wide gene-by-gene approach was applied to study the epidemiology of ESBL-*E. coli* and VRE.

Results; A total of 34 isolates from 27 patients (6.1%) admitted to Dutch hospitals were ESBL/pAmpC positive and 29 ESBL-*E. coli*, three pAmpC-*E. coli,* one ESBL-*E. cloacae* and one pAmpC-*P. mirabilis* were found. In the German hospital, 18 isolates (16 *E. coli* and 2 *K. pneumoniae*) from 17 patients (7.7%) were ESBL positive. In isolates from the hospitalized patients CTX-M-15 was the most frequently detected ESBL-gene. In the Dutch community, 11 individuals (2.75%) were ESBL/pAmpC positive: 10 ESBL *E. coli*, (CTX-M-1 being the most prevalent gene) and one pAmpC *E. coli*. Six Dutch (1.3%) and four German (3.9%) hospitalized patients were colonized with VRE. Genetic relatedness by core genome multi-locus sequence typing (cgMLST) was found between two ESBL- *E. coli* isolates from Dutch and German cross-border hospitals and between VRE isolates from different hospitals within the same region.

Conclusions; The prevalence of ESBL/pAmpC-*Enterobacteriaceae* was similar in hospitalized patients across the Dutch-German border region, whereas VRE prevalence was slightly higher on the German side. The overall prevalence of the studied pathogens was lower in the community than in hospitals in the Northern Netherlands. Cross-border transmission of ESBL-*E. coli* and VRE seems unlikely based on cgMLST analysis, though continuous monitoring is necessary to keep the epidemiology of resistant pathogens updated thereby helping to control their spread.

INTRODUCTION

International travel and patient care are risk factors for dissemination of bacteria including multidrug-resistant microorganisms (MDRO), such as Extended spectrum β-lactamase (ESBL) and carbapenemase (CP)-producing *Enterobacteriaceae* [1, 2], and vancomycin resistant enterococci (VRE). The prevalence of the latter has increased in the last years due to successful polyclonal subpopulations of hospital associated (HA) *E. faecium* (previously designated clonal complex CC17) and which are also associated with amoxicillin resistance (ARE) [3]. These populations are distinct from *E. faecium* isolates in the community and isolates from non-human sources [4, 5].

The Netherlands and Germany as bordering countries with possible transfer of patients between them, created a cooperative network to prevent the spread of MDRO and to harmonize guidelines in healthcare settings [1, 6]. Surveillance studies to monitor the prevalence, resistance patterns and molecular background of MDRO in hospitals and the community are essential to get insights into their epidemiology to implement infection prevention measures. Bacterial whole-genome sequencing (WGS) has been demonstrated to be very useful for epidemiological surveillance and detection of antimicrobial resistance [7]. The gene-by gene approach uses a defined set of genes to extract an allele-based profile which makes it scalable and portable between laboratories [8, 9]. A core genome multilocus sequence typing (cgMLST) scheme has been developed for *E. faecium* to distinguish between epidemiologically related and unrelated isolates [10]. Although there is no cgMLST scheme nor threshold publically approved yet for *E. coli*, there are several tools available that allow to define an *ad hoc* cgMLST.

The aim of this study was to perform a point-prevalence study on ESBL/plasmid mediated AmpC β-lactamase (pAmpC)/CP- *Enterobacteriaceae* and HA *E. faecium* (VRE and ARE) in hospitals in the Northern Dutch-German border region and to determine the predominant resistance genes. In addition, stool community samples from the Northern Netherlands were screened for the same resistant pathogens. A cgMLST was used to study hospital and cross border dissemination of ESBL-*E. coli* and VRE.

MATERIALS AND METHODS

Study design

A prospective point prevalence study was conducted in four of the largest hospitals (in total 3550 beds) in the Northern Netherlands between November 2012 and February 2013, covering a total population of approximately 2.85 million people. The Hospital Ethical Committee of the University Medical Center Groningen (UMCG) was informed and patients were approached to voluntarily participate in the study. Patients included in this study provided their written informed consent and a questionnaire concerning epidemiological and clinical data. The following high-risk wards for antibiotic resistant microorganisms were selected: intensive care units (ICU), vascular surgery, internal medicine haematology/oncology and dialysis wards (both for in- and outpatients). Gynaecology and neurology (low-risk wards) were also included for comparison. From the largest German university hospital in the same (border) region, patients from four ICUs, a surgical ward and a haematology/oncology ward were screened during October and November 2012 and included in the study. After consent agreement, all admitted patients from the studied wards were screened until completing a minimum of 100 samples per hospital.

The study in healthy people living in the the Northern Netherlands was conducted retrospectively, using control patients included in a previous case-control study on microorganisms causing gastroenteritis. Control subjects were patients attending their general practitioner for a variety of medical questions, but no gastrointestinal problems, in the period between August 2010 and December 2012 [11]. No prevalence study was performed in the community in Germany.

Sample collection

A total of 445 rectal swabs (Copan ESwab™) were taken from hospitalized patients (median age = 66 years, range 18-99 years) in the Northern Netherlands, 51.7% (n=230) from men and 48.3% (n=215) from women. A total of 328 (73.7%) patients were screened at high risk wards and 117 (26.3%) patients were screened at low risk wards (Table 1). In the German university hospital 242 patients (median age = 64 years, range 0-94 years) were included, 64.5% (n=156) men and 35.5% (n=86) women. Of these 242 patients, 140 were screened only for ESBL, 22 only for VRE and 80 for both. From the Dutch community study, 400 frozen faeces samples were included; 41% (n=164) from men, and 59% (n=236) from women, 12% of the samples were from children. The median age of the healthy individuals was 47.5 years (range 0-84 years).

Table 1: Distribution of ESBL/pAmpC producing *Enterobacteriaceae*, and amoxicillin and vancomycin resistant *E. faecium* among the different wards in Dutch hospitals.

Ward	ESBL/pAmpC producing *Enterobacteriaceae*	Amoxicillin resistant *E. faecium*	Vancomycin resistant *E. faecium*
High risk (n=328)	19 (5.8 %)	99 (30.2%)	6 (1.8%)
– Intensive care unit (n=102)	6 (5.9%)	31 (30.4%)	1 (1%)
– Vascular surgery (n=54)	6 (11.1%)	15 (27.8%)	1(1%)
– Internal medicine hematology/oncology (n=81)	1 (1.2%)	36 (44.4%)	2 (2.5%)
– Dialysis (n=91)	6 (6.6%)	17 (18.7%)	2 (2.2%)
Low risk (n=117)	8 (6.8%)	6 (5.1%)	0 (0%)
– Gynaecology (n=55)	3 (5.5%)	1 (1.8%)	0 (0%)
– Neurology (n=62)	5 (8.1%)	5 (8.1%)	0 (0%)
Total (n= 445)	27 (6.1%)	105 (23.6%)	6 (1.3%)

MICROBIOLOGICAL DETECTION, IDENTIFICATION AND SUSCEPTIBILITY TESTING

Dutch hospitals and retrospective Dutch community study

Rectal swabs (Dutch hospitalized patients) and approximately 50µg of faeces per sample (Dutch community patients) were enriched in selective broths: VRA broth containing BHI (brain heart infusion) with 20 mg/L amphoterin-B, 20 mg/L aztreonam, 20 mg/L colistin and 16mg/L amoxicillin and TSB-VC broth containing tryptic soy broth with 8 mg/L vancomycin and 0.25 mg/L cefotaxim. Both broths were incubated for 24h at 35 °C +/-1°C. Subsequently, 10µL of VRA broth was subcultured on VRE Brilliance agar (Oxoid®) and BMEG-2 agar (blood agar containing 64 mg/L meropenem, 2 mg/L gentamicin, 10 mg/L oxacillin and 20 mg/L amphotericin-B) for identification of VRE and all ARE, respectively. Ten µL of TSB-VC broth was subcultured onto ME/CF/CX comparted plates, containing iso-sensitest agar with 1 mg/L meropenem, 1 mg/L ceftazidim, or 1 mg/L cefotaxim respectively, plus 20 mg/L vancomycin and 20 mg/L amphotericin-B (Mediaproducts, Groningen), for selection of ESBL/pAmpC/CP- producing bacteria. Plates were incubated for 24h at 35°C +/-1°C, except for VRE Brilliance agar plates that were incubated for 48h.

Suspected colonies on VRE Brilliance, BMEG-2 and ME/CF/CX agar plates were streaked on blood agar (one isolate per morphotype). Species identification was done by Matrix-assisted Laser Desorption/Ionization Mass Spectrometry (MALDI-TOF) (Bruker Daltonik

GmbH, Bremen). Confirmed *Enterococcus* spp and *Enterobacteriaceae* spp, were tested for antibiotic susceptibility using VITEK®2 (bioMérieux) automatic system and EUCAST clinical breakpoints.

German hospital

Rectal swabs were directly plated on chromID® ESBL agar (bioMérieux) for ESBL screening and enriched Enterococcosel™ Broth (Bile Esculin Azide Broth) (BD; Becton, Dickenson and Company) was used for VRE screening and subsequently cultured on chromID® VRE agar (bioMérieux).

Species identification and antibiotic susceptibility testing was done by MALDI-TOF (Bruker Daltonik GmbH, Bremen) and VITEK®2 (bioMérieux), respectively, following EUCAST criteria. Confirmation of ESBL was performed using disk diffusion (cefotaxime 30 µg, cefotaxime 30 µg plus clavulanic acid 10 µg, ceftazidime 30 µg, ceftazidime plus clavulanic acid 10 µg, cefepime 30 µg, cefepime 30 µg plus clavulanic acid 10 µg, and cefoxitin 30 µg) (Mast Diagnostics, Derby Road, Bootle, UK).

PCRs and microarray

Enterococci isolates from The Netherlands were screened by in-house PCR for *IS16* (a marker for specific hospital associated strains), *vanA* and *vanB* genes as described previously [12, 13]. The GenoType Enterococcus (Hain Lifescience GmbH) was used in enterococci isolates from Germany, which detects species and genotypes *vanA, vanB, vanC1 and vanC2*. ESBL and VRE positive isolates were sent to our hospital for further characterization.

Enterobacteriaceae isolates resistant to third generation cephalosporins and natural chromosomal AmpC producers intermediate or resistant to cefepime were selected for DNA extraction using the UltraClean Microbial DNA Isolation Kit (MoBio, Laboratories, Inc.) and further characterized for the presence of ESBL/AmpC genes using a DNA-array (Check-MDR CT103, Check-points, Wageningen, The Netherlands) [14].

Whole-genome sequencing of VRE and ESBL-*E. coli*

Whole-genome sequencing (WGS) was performed for all ESBL-*E. coli* and VRE isolates. For each isolate, several colonies (about 5 µl) of the culture were suspended in 300 µl microbead solution, which was subjected to DNA extraction with the Ultraclean Microbial DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA, USA). The DNA concentration and purity were measured using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA,

USA) and the Qubit double-stranded DNA (dsDNA) HS and BR assay kits (Life Technologies, Carlsbad, CA, USA). One nanogram of bacterial DNA was used for library preparation. The DNA library was prepared using the Nextera XT library preparation kit with the Nextera XT v2 index kit (Illumina, San Diego, CA, USA). The library fragment length was aimed at fragments with a median size of 575 bases and was assessed with the Genomic DNA ScreenTape assay with the 2200 TapeStation system (Agilent Technologies, Waldbronn, Germany). Subsequently, the library was sequenced on a MiSeq sequencer, using the MiSeq reagent kit v2 generating 250-bp paired-end reads. Sequencing was aimed at a coverage of at least 60-fold. MiSeq data were processed with MiSeq control software v2.4.0.4 and MiSeq Reporter v2.4 (Illumina, San Diego, CA, USA). Reads were quality-trimmed using the CLC Genomics Workbench software version 9.0.1 (CLC bio, Aarhus, Denmark) using default settings except for the following modifications: "trim using quality scores was set to 0.02" and "discard reads below length was set to 15". Subsequently, trimmed-reads were *de novo* assembled with an optimal word size of 29 and a minimum contig length of 500. Metrics on raw read and assembly level are provided in Table S1.

Core genome multi locus sequence typing (cgMLST) of VRE and ESBL-*E. coli*

A genome wide gene-by-gene comparison approach was used to determine the genetic relatedness using SeqSphere⁺ version 3.4.0 (Ridom GmbH, Münster, Germany) [8]. Genome assemblies from the VRE isolates were analyzed using the *E. faecium* cgMLST scheme previously published, considering a cluster alert distance of 20 different alleles [10].

An *ad hoc* cgMLST and whole genome MLST (wgMLST) scheme was determined for *E. coli* isolates using the MLST⁺ target definer function with default parameters [15] and *Escherichia coli* K-12 as a reference (GenBank accession no. NC_010473.1). The filters applied to reference genome were: "minimum length filter" that discards genes shorter than 50 bases; "start codon filter" that discards all genes that contain no start codon at the beginning of the gene: "stop codon filter" that discards all genes that contain no stop codon, more than 1 stop codon or if the stop codon is not at the end of the gene: "homologous gene filter" that discards all genes that have fragments that occur in multiple copies in a genome (with identity ≥ 90% and more than 100 bases overlap); "gene overlap filter" that discards the shorter of two overlapping flanking genes if these genes overlap > 4 bp. The remaining genes were then used in a pairwise comparison using BLAST [8] with 45 query genomes (Table S2a). All genes of the reference genome that were common in all query genomes with a sequence identity of ≥90% and 100% overlap, and with the default parameter stop codon

percentage filter turned on, formed the final cgMLST scheme; this discards all genes that have internal stop codons in >20% of the query genomes. Additionally, 26 plasmid sequences (Table S2b) were added to exclude such genes are part of the cgMLST typing scheme. The final cgMLST scheme consisted of 1.771 targets/genes, and 2329 accessory genes were additionally included for the wgMLST scheme (Table S3 and S4). The minimum coverage of the genome assemblies was 20 times (Table S1) and the percentage of good reads included in the cgMLST were 97.6% for *E. coli* and 98.6 for *E. faecium* (Table S5 and S6).

Furthermore, to determine the genetic relatedness, the genetic distance for the *E. coli* isolates was calculated as the proportion of allele differences: dividing the number of allele differences between two genomes by the total number of genes commonly shared by those two genomes [16]. In this study thresholds for genetic distance were described to discriminate between epidemiologically related and unrelated *E. coli* isolates as 0.0095 when using wgMLST and 0.0105 for cgMLST.

E. coli STs were determined uploading genome assemblies to SeqSphere+ software following the scheme of Wirth et al [17]. Sequence genomes with no conclusive results for the 7-gene MLST were uploaded to the Enterobase database [18]. Additionally, *E. coli* major phylogenetic groups (A, B1, B2 and D) were analysed *in silico* by using MLST+ Target Definer function of SeqSphere+, including the *chuA*, *yjaA*, and TSPE4.C2 loci [19].

Genome assemblies were also uploaded to the Center for Genomic Epidemiology to extract information on resistance genes (ResFinder) and virulence factors (VirulenceFinder), and species confirmation for VRE and ESBL-*E.coli* (KmerFinder), and serotype (SerotypeFinder) and plasmid replicons (PlasmidFinder) for ESBL-*E.coli* [20-25].

STATISTICAL ANALYSIS

In the Dutch hospital prevalence study, associations between ESBL and ARE carriage and the following variables were analyzed: length of hospital stay, antibiotic use and (low or high risk) ward. Information was gathered by the questionnaires. Statistical analyses were performed using SPSS for Windows, v. 20.0. Univariate analyses were performed using the Fisher's exact or Chi-square methods for categorical variables. The Mann-Whitney U test was used as a non-parametric tests in variables with no normal distribution. Results with a *p*-value of ≤0.05 were considered to be statistically significant. All *p*-values are two-tailed.

RESULTS

Extended-spectrum β-lactamase (ESBL)/plasmid AmpC (pAmpC)-producing *Enterobacteriaceae*

Thirty-four isolates from 27 of the 445 included patients admitted to hospitals in the Northern Netherlands (6.1%) were confirmed ESBL and/or pAmpC positive. A total of 85.2% (23/27), 14.8% (4/27) and 3.7% (1/27) of these patients were positive for ESBL, pAmpC and both, respectively. Among the 34 isolates, 32 were *E. coli,* of which 29 were ESBL positive and three were pAmpC producers. Resistance genes detected in the *E. coli* isolates are shown in Table 2. CTX-M-15 (n=8) and CTX-M-14 (n=8) were the most prevalent ones. The other two isolates were an *E. cloacae,* containing a CTX-M-1-like gene and a pAmpC CMY-II producing *P. mirabilis.* At high risk wards, 19 patients (5.8%) were found with ESBL/pAmpC isolates compared to 8 patients (6.8%) at low risk wards (p=0.68; NS). No association was found between ESBL/pAmpC carriage and antibiotic use, length of hospital stay or ward (Table 1).

In the German hospital, a total of 18 isolates from 17 patients (17/220; 7.7%) were ESBL positive. Sixteen isolates were *E. coli* and two were *K. pneumoniae.* Of these, twelve *E. coli* and one *K. pneumoniae* isolates were available for molecular testing. Six out of twelve (50%) *E. coli* isolates and the *K. pneumoniae* isolate had a CTX-M-15 gene (Table 2).

In the retrospective Dutch community study, 11 patients (11/400; 2.75%) were ESBL/pAmpC positive: 10 ESBL *E. coli*, (CTX-M-1 being the most prevalent gene) and one pAmpC *E. coli.* (Table 2). Overall, no carbapenem resistance was observed neither in the community nor in the hospitals.

E. coli MLST and phylogenetic groups

Among ESBL/pAmpC- *E. coli* isolates from Dutch hospitals, the most prevalent STs were ST131 (clonal complex (CC) ST131; n=5, 15.6%), all of them belonging to phylogroup B2 (Table 2). In the Dutch community isolates 10 different STs were found, most of them belonging to CC ST10 (n=3, 27.3%) and one isolate to ST131 (phylogroup B2). In the German hospital, the most prevalent STs were ST38 (33.3%) and ST10 (33.3%) (Table 2).

Table 2: Molecular characterization of the *E. coli* isolates from the community and hospital patients in The Netherlands and Germany.

Sample[1]	Hospital/ Ward	β-lactamase genes	Phylogroup	ST	CC
Community					
1_Esco_CA-NL		blaCTX-M-1, blaTEM-1B	B2	131	ST131
2_Esco_CA-NL		blaSHV-12	B2	117	none
3_Esco_CA-NL		blaCMY-2	D	2309	none
4_Esco_CA-NL		blaCTX-M-1	D	57	ST350
5_Esco_CA-NL		blaCTX-M-1, blaTEM-1B	A	10	ST10
6_Esco_CA-NL		blaCTX-M-1, blaTEM-1B	B1	1079	none
7_Esco_CA-NL		blaCTX-M-1, blaTEM-1B	A	10	ST10
8_Esco_CA-NL		blaCTX-M-15	D	648	ST648
9_Esco_CA-NL		blaCTX-M-15	A	617	ST10
10_Esco_CA-NL		blaCTX-M-15	A	1312	none
11_Esco_CA-NL		blaCTX-M-14b, blaTEM-1B	D	38	ST38
Hospital					
12_Esco_HA-NL	A/ Gynaecology	blaCTX-M-15, blaTEM-1B	D	5463	none
12b_Esco_HA-NL	A/ Gynaecology	blaCTX-M-15, blaTEM-1B	D	5463	none
13_Esco_HA-NL	A/ Neurology	blaCTX-M-27	B2	131	ST131
14_Esco_HA-NL	A/ Dialysis outpatient	blaCTX-M-15, blaTEM-1B	A	93	ST168
15_Esco_HA-NL	A/ ICU	blaCMY-2, blaTEM-1B	D	354	ST354
16_Esco_HA-NL	A/ ICU	blaCTX-M-15, blaTEM-1B, blaOXA-1	B1	58	ST155
17_Esco_HA-NL	A/ ICU	blaCTX-M-15, blaTEM-1B	B1	38	ST38
18_Esco_HA-NL	A/ ICU	blaTEM-52C	B1	453	ST86
19_Esco_HA-NL	A/ ICU	blaCTX-M-1	B1	641	ST86
20_Esco_HA-NL	A/ ICU	blaSHV-12	A	5888	none
20b_Esco_HA-NL	A/ ICU	blaCTX-M-1	B1	58	ST155
21_Esco_HA-NL	B/ Gynaecology	blaCTX-M-14	B1	101	ST101
22_Esco_HA-NL	B/ Dialysis outpatient	blaCTX-M-14	B1	38	ST38
22c_Esco_HA-NL	B/ Dialysis outpatient	blaCTX-M-14	D	38	ST38
23_Esco_HA-NL	B/ Vascular surgery	blaCMY-2, blaTEM-1B	D	1508	none
24_Esco_HA-NL	B/ Neurology	blaTEM-52C	D	2064	none
25_Esco_HA-NL	B/ Neurology	blaCTX-M-3, blaTEM-1B	B2	95	ST95
25b_Esco_HA-NL	B/ Neurology	blaCTX-M-3, blaTEM-1B	D	95	ST95
26_Esco_HA-NL	C/ Gynaecology	blaCTX-M-15, blaOXA-1	B2	131	ST131
27_Esco_HA-NL	C/ Dialysis outpatient	blaCTX-M-1, blaTEM-33	A	3478	none
28_Esco_HA-NL	C/ Dialysis outpatient	blaCTX-M-14	A	10	ST10
29_Esco_HA-NL	C/ Neurology	blaCTX-M-1	B1	603	none
30_Esco_HA-NL	C/ Vascular surgery	blaCTX-M-14	A	410	ST23
31_Esco_HA-NL	D/ Vascular surgery	blaCTX-M-14, blaTEM-1B, blaOXA-1	B1	58	ST155

Sample[1]	Hospital/ Ward	β-lactamase genes	Phylogroup	ST	CC
32_Esco_HA-NL	D/ Vascular surgery	blaCTX-M-1	D	117	none
32b_Esco_HA-NL	D/ Vascular surgery	blaDHA-1, blaTEM-1B	B2	131	ST131
33_Esco_HA-NL	D/ Vascular surgery	blaCTX-M-14	D	69	ST69
33b_Esco_HA-NL	D/ Vascular surgery	blaCTX-M-14	D	69	ST69
34_Esco_HA-NL	D/ Internal medicine	blaCTX-M-55, blaOXA-1	B1	4385	none
35_Esco_HA-NL	D/ Dialysis outpatient	blaCTX-M-15, blaTEM-1B, blaOXA-1	B2	131	ST131
35b_Esco_HA-NL	D/ Dialysis outpatient	blaCTX-M-15, blaOXA-1	B2	131	ST13
36_Esco_HA-NL	D/ Dialysis outpatient	blaCTX-M-1, blaTEM-1B	B1	58	ST 155
37_Esco_HA-DE	ICU 1	blaCTX-M-15	D	38	ST38
38_Esco_HA-DE	ICU 6	blaCTX-M-14	D	38	ST38
39_Esco_HA-DE	ICU 2	blaCTX-M-14	A	10	ST10
40_Esco_HA-DE	ICU 6	blaCTX-M-15, blaTEM-1B, blaOXA-1	B1	448	ST448
41_Esco_HA-DE	Surgical ward	blaCTX-M-1, blaTEM-1B	A	10	ST10
42_Esco_HA-DE	Haemato-oncology ward	blaCTX-M-15, blaTEM-1B, blaOXA-1	A	90	ST23
43_Esco_HA-DE	ICU 4	blaCTX-M-15, blaOXA-1	A	34	ST10
44_Esco_HA-DE	ICU 3	blaTEM-187	A	10	ST10
45_Esco_HA-DE	ICU 3	blaCTX-M-15, blaOXA-1	D	38	ST38
46_Esco_HA-DE	ICU 3	blaCTX-M-1, blaTEM-1B	A	10	ST10
47_Esco_HA-DE	ICU 1	blaCTX-M-15	D	38	ST38
48_Esco_HA-DE	ICU 1	blaCTX-M-14, blaTEM-1B	D	1177	--

[1]CA: community acquired; HA: hospital acquired; NL: The Netherlands; DE: Germany; numbers refer to individual patients and a letter behind a number indicates that more than one isolate was obtained from the patient

Table 3: Variables associated with carriage of amoxicillin-resistant *E. faecium* (ARE)

Variables	ARE n=105	No ARE n=340	*p*-value*	ESBL/pAmpC n=27	No ESBL/pAmpC n=418	p-value *
Hospitalization days median (range)	12 (1-127)	3 (1-107)	*p*<0.001	4 (1-127)	4 (1-36)	*p*=0.886
Ward			*p*<0.001			*p*=0.657
– High risk (n=328)	99 (94.3%)	229 (67.4%)		19 (70.4%)	309 (73.9%)	
– Low risk (n=117)	6 (5.7%)	111 (32.6%)		8 (29.6%)	109 (26.1%)	
Antibiotic use (n=145)	62 (59%)	83 (24.4%)	*p*<0.001	7 (25.9%)	138 (33%)	*p*=0.529
– Penicillins **	26 (24.8%)	29 (8.5%)	*p*<0.001	3 (11.1%)	35 (8.4%)	*p*=0.494
– Fluoroquinolones	28 (26.7%)	15 (4.4%)	*p*<0.001	1 (3.7%)	42 (10%)	*p*=0.499
– 3rd gen cephalosporins	11 (10.5%)	19 (5.6%)	*p*=0.081	1 (3.7%)	29 (6.9%)	*p*=1.00

*Results with a *p*-value of ≤0.05 were considered to be statistically significant. All *p*-values are two-tailed.

**used penicillins: benzylpenicillin, flucloxacillin, amoxicillin-clavulanic acid and piperacillin-tazobactam.

Ampicillin and vancomycin resistant *E. faecium* (ARE and VRE)

In the Dutch hospitals 105 patients (105/445; 23.6%) were colonized with ARE, including six patients (6/445; 1.3%) with VRE. All ARE were positive for *IS16* and all VRE were *vanB* positive. Colonization of ARE (and VRE) was associated with high risk wards (p<0.001), prolonged hospitalization (p<0.001) and use of antibiotics (p=0.05), especially penicillins and fluoroquinolones (p<0.001) (Table 3).

In the border German university hospital four (4/102; 3.9%) VRE isolates were isolated. Three of them were *vanA* positive and one was *vanB* positive.

In the retrospective Dutch community study, six ARE (6/400; 1.5%) were found, three of them were *IS16* positive. Only one *vanA*-VRE (1/400; 0.25%) was found, this strain was ampicillin susceptible and *IS16* negative.

cgMLST and wgMLST comparison of ESBL-*E. coli* isolates from the community and hospitals

Genome assemblies of 55 ESBL-*E. coli* (Dutch community (n=11), Dutch hospitals (n=32) and German hospital (n=12)) of this study were analyzed by a gene-by-gene approach and the allelic distance from the cgMLST and wgMLST were visualized in a minimum spanning tree (Figure 1 and Figure S1, respectively).

Six groups of isolates with a lower number of different alleles (≤ 35) by cgMLST were further analyzed. Table S7 summarizes the origin of the isolates in every group and the core and whole genome genetic distance. Those groups formed by isolates with an epidemiological link (isolated from the same patient; group 1, 4, 5a, 6a and 7), showed a core and whole genome genetic distance lower than 0.0030 and 0.0046, respectively. In addition, isolates of group 5b, although with unknown epidemiological link, had a core genetic distance of 0.0063 and a whole genome genetic distance of 0.0076. Both isolates were positive for CTX-M-14, however no plasmid replicons were found in one of them (isolate 38_Esco_HA-DE) (Table S7).

Among those groups including isolates with non (or unknown) epidemiological link, the core genome genetic distance was between 0.0122-0.0199 and the whole genome genetic distance was between 0.0104-0.0208 (groups 2, 3, 6b, and 6c; Figure 1). Resistance and virulence profiles of the isolates are shown in Table S8.

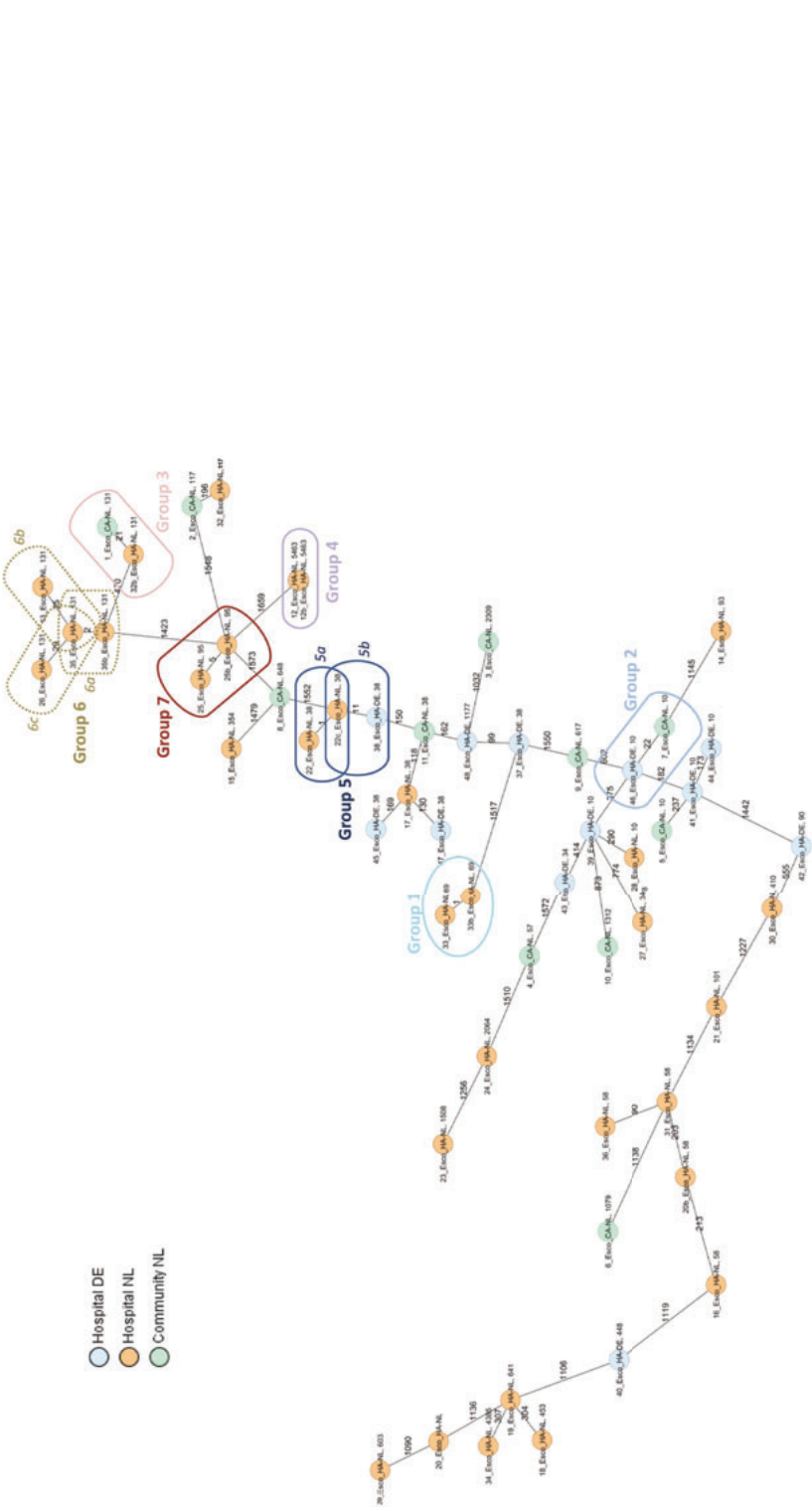
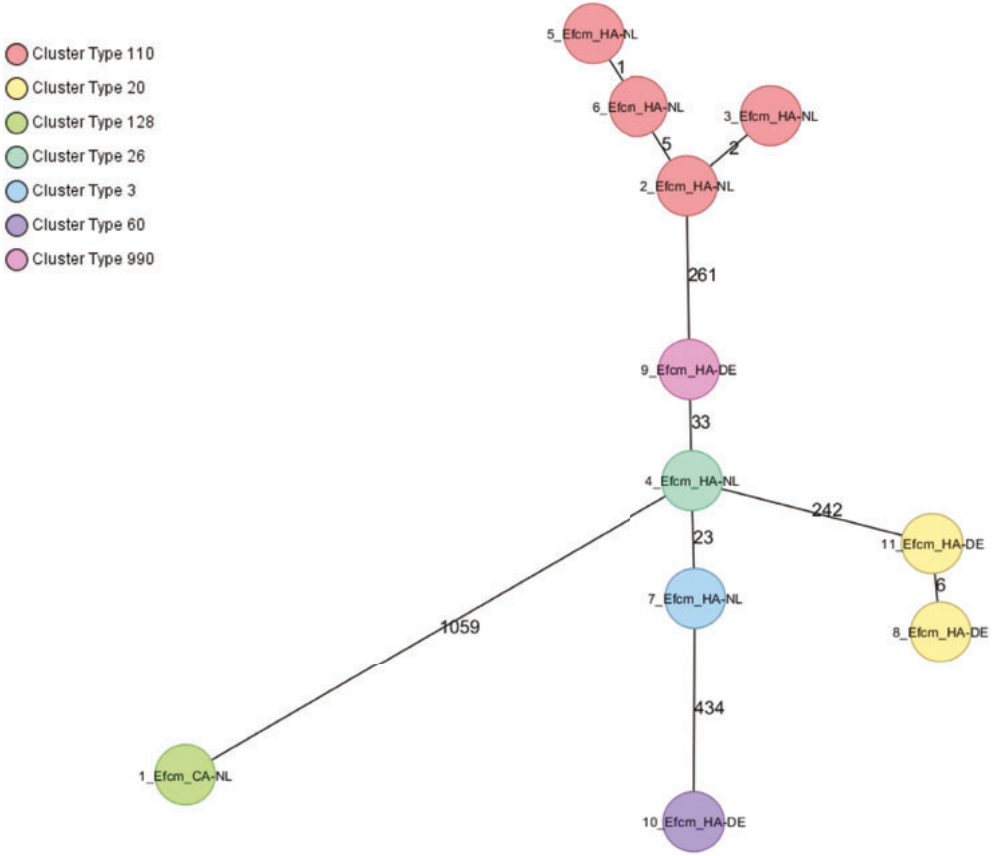


Figure 1: Minimum spanning tree of ESBL-*E. coli* isolates from hospitals and the community. Distance based on a cgMLST of 1771 genes using the parameters "pairwise ignoring missing values" during calculation. Each circle represents a genotype, colors indicate geographical origin and community or hospital. Orange: hospital-The Netherlands; blue: hospital-Germany; green: community-The Netherlands. Number of different alleles are indicated on the edges between connected isolates (nodes). The cut-off values for defining a group was 35 alleles. Isolates are presented by their ID and ST.

Figure 2: Minimum spanning tree of VREfcm, cgMLST based on 1423 genes using the parameters "pairwise ignoring missing values" during distance calculation. Each circle represents a genotype and colors indicate cluster types (CT). Number of different alleles are indicated on the edges between connected isolates (nodes). Isolates are presented by their ID, ST and CT.



cgMLST comparison of VRE isolates from the community and hospitals

A minimum spanning tree was created for the 11 VRE isolates (Dutch community (n=1), Dutch hospitals (n=6) and German hospital (n=4)). Two clusters of isolates from different patients were observed (Figure 2). One cluster of four *vanB*-VRE isolates from the Dutch hospital belonged to Cluster Type (CT) 110 (ST17); two isolates were from the same ward in hospital A and the other two isolates were isolated from different wards in hospital B. The other cluster of two *vanA*-VRE isolates were isolated from different wards from the German hospital (CT 20, ST203). The resistance and virulence genotypes of VRE isolates are shown in Table S8.

Nucleotide sequence accession number.

Sequence data obtained in this study has been deposited at the National Center for Biotechnology Information under BioProject no. PRJNA352198.

DISCUSSION

This study shows the molecular epidemiology of ESBL/pAmpC and HA *E. faecium* in hospitals in the Northern Dutch-German border region and the community in the Northern Netherlands. Dutch hospitals showed a prevalence for ESBL/pAmpC, VRE and ARE of 6.1%, 1.3% and 23.6% respectively, whereas the prevalence in the community was 2.75%, 0.25% and 1.5%, respectively. The German hospital had an ESBL/pAmpC prevalence of 7.7% and 3.9% for VRE.

A previous study reported a prevalence of ESBL- producing bacteria of 4.9% in the Netherlands [26], comparable to the 6.1% prevalence observed in Dutch hospitals in this study. A prevalence of 5.6% ESBL- producing *E. coli* isolates in hospitalized and ambulatory patients in Germany has been reported recently [27], which is slightly lower than the 7.7% observed in the present study.

Furthermore, we observed an ESBL- *E. coli* prevalence of 2.5% in the Northern Netherlands community, which is low compared to previous studies in other regions, in which the prevalence in the community ranged from 4.7% (2009) to 10.1% (2011) [28, 29]. This difference may have several reasons. First, ESBL prevalence may vary between regions and over time, and natural eradication of resistant *Enterobacteriaecae* might occur over time in the community [30]. Additionally, samples included in this study were only chosen from patients without any gastrointestinal complaints, a factor which otherwise has been described to be associated with high ESBL prevalence [28].

The majority of the resistance genes found in our community isolates were CTX-M-1 which is broadly disseminated among animals in Europe, especially in cattle and pigs, followed by the CTX-M-15 gene, commonly associated with human origin [27, 28]. The latter was the most frequent gene among the Dutch and German hospital isolates, in concordance with previous studies [27, 28, 31].

The pAmpC prevalence in *E. coli* in our study was 0.3%, comparable to the prevalence of 0.6% what was reported in the study of van Hoek et al. [29] (0.6% pAmpC *Enterobacteriaceae*) and somewhat lower to findings of Reuland et al. (1.3% pAmpC- *E.coli)* [32]. The most common pAmpC gene found in hospital and community isolates were CMY-II, which is together with DHA frequently found in human isolates [32].

ESBL-producing *E. coli* belonging to clonal complex ST131-phylogroup B2 are usually associated with more virulent strains [33]. These were frequently found in the Dutch hospitals included in the present study but only sporadically in the community samples. This CC ST131-phylogroup B2 was also prevalent in a study carried out in hospitals in the Rotterdam region [34]. CC ST10 was predominant among the ESBL- producing *E. coli* in the community, the same clonal complex was also described to be prevalent in another Dutch study in community patients [28].

We observed an overall ARE and VRE prevalence in hospitalized patients of 23.6% and 1.3%, respectively. Similar observations were made in a study performed in Dutch hospitals in 2008 reporting ARE carriage rates of 10-16% upon admission and 15-39% on acquisition in haematology and gastroenterology/nephrology wards [35]. The clinical significance of enterococcal infections and active VRE screening has been a matter of discussion. However, in immunocompromised patients, high morbidity and mortality rates have been reported in infections caused by enterococci [36] . In this study ARE/VRE carriage was associated with prolonged hospitalization and antibiotic use, which is in line with previous literature [37]. We found a high carriage rate of ARE in high risk wards (30.2%). Notably, these patients may be at risk for a subsequent infection. Since 2011, VRE started to become a problem in multiple hospitals in the Netherlands: a total of fourteen hospitals were affected with outbreaks of VRE in October 2012 [38]. However, in this study a prevalence of VRE (*vanB*) carriage of only 1.3% was found. This is probably due to extensive infection prevention measures and successful outbreak management control. The prevalence of 1.3% is similar to what has been previously published in the Netherlands, with prevalence rates ranging from 1.4%- 2% in the 90s [39, 40]. The VRE prevalence in the German hospital was slightly higher (3.9%),

though it is known that Germany has a higher VRE prevalence compared to the Netherlands [41].

In our Dutch community one *vanA*-VRE was found, that was ampicillin susceptible and *IS16* negative, indicative for a non-hospital origin [4, 5]. Endtz *et al.* reported a higher number of VRE in the community (2%), however this study did not include information about ampicillin resistance nor *IS16* which makes it difficult to determine if they had a hospital or non-hospital origin [4, 5].

The cgMLST analysis in our study showed heterogeneity among *E. coli* species, and isolates were genetically distributed independently of their origin. The hospital and community ESBL-*E. coli* isolates included in this study did not show any genetic relatedness except for the ones isolated from the same patient and for two isolates (group 5b) from patients in different hospitals across the Dutch-German border, in a distance of approximately 200km and with no known epidemiological link. The patient from the Dutch hospital was a dialyses outpatient (isolation date December 2012) whereas the patient from the German hospital was admitted to ICU (isolation date November 2012). Interestingly, both isolates harbored the same ESBL gene and virulence factors.

Genetic relatedness was found between four VRE isolates (CT110) from patients from two different Dutch hospitals (Figure 2), which indicates transmission between wards, but also between hospitals in a close geographical region similar to findings of a previous population-based study of VRE using WGS that also showed intra- and inter-regional spread of closely related VRE isolates [42]. Although no genetic relatedness was found between VRE isolates of Dutch and German hospitals, the numbers of VRE isolates were too low to draw definite conclusions. It is known that several VRE cluster types co-circulate in Germany and the Netherlands (data not shown). However, only some laboratories have implemented the use of cgMLST in their routine to analyse VRE outbreaks and more epidemiological studies are needed to investigate cross-border transmission of VRE.

To our knowledge there are no similar studies that compare and investigate the molecular epidemiology of ESBL *E.coli* and VRE in hospitals and the community by WGS. Recently, the same approach has been used to study the clonality of ESBL- producing *Enterobacteriaceae* from environmental and stool samples from farmers suggesting possible cross-transmission between the farmers and the environment. This was only based on number of allele differences [16, 43] which makes it difficult to interpret results without considering the total number of genes included in the cgMLST scheme. In our study, we determined the genetic relatedness between ESBL-*E. coli* using cgMLST or wgMLST comparison and genetic

distance calculation. These results were in concordance with the genetic distance thresholds of 0.0095 (wgMLST) and 0.0105 (cgMLST) previously established for *E. coli* based on known existing epidemiological links by analysing more than 2.000 ESBL-*Enterobacteriaceae* isolates from Dutch hospitals [16].

In another study, a cgMLST approach for several MDR bacteria was prospectively used for taking relevant infection control decisions in a hospital setting [44]. A threshold of >10 differing alleles was defined to exclude nosocomial transmission of MDR *E. coli* [44]. If we would have applied this threshold we would have missed the genetic relatedness between isolates belonging to group 5b, presenting 11 different alleles (Figure 1 and Table S7). This highlights that thresholds based on number of allele differences are only applicable to specific collections within a study, whereas the genetic distance calculation seems to give a more objective result, independently of the analysed population.

We acknowledge this study has some limitations. No community study in the German cross-border region, neither ARE monitoring in the German hospital were performed. Laboratory methods for isolation of ESBL *Enterobacteriaceae* and VRE differed between Dutch and German hospitals since no enrichment broth was used in Germany, however selective media agar was used in both regions. Since this study was anonymous, some epidemiological data were not available which makes it more difficult to draw conclusions regarding genetic relatedness among isolates between patients.

In conclusion, the results of this study suggest that ESBL/pAmpC-*E.coli* circulate in the hospital and the community, although a higher prevalence of ESBL/pAmpC-*E. coli* was observed in hospitals compared to the community in the Northern Netherlands. Hospitals in the Netherlands and Germany showed a similar prevalence of ESBL/pAmpC-*Enterobacteriaceae*. VRE prevalence was still low in the hospital as well as in the community in the Northern Netherlands. The German hospital showed a slightly higher VRE prevalence compared to hospitals in the Northern Netherlands. Nosocomial but no cross-border transmission of VRE was observed in this study. Epidemiologically related ESBL-*E. coli* and VRE were uncommon across the Dutch-German border in the studied population, as only two ESBL- *E. coli* isolates from a Dutch and a German hospital were genetically similar. Cooperation between bordering countries and continuous monitoring using high discriminatory typing methods are still necessary to keep the epidemiology of resistant pathogens updated thereby helping to control their spread.

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Transparency declarations

None to declare

REFERENCES

1. Muller J, Voss A, Kock R, Sinha B, Rossen JW, Kaase M, Mielke M, Daniels-Haardt I, Jurke A, Hendrix R, Kluytmans JA, Kluytmans-van den Bergh MF, Pulz M, Herrmann J, Kern WV, Wendt C, Friedrich AW: Cross-border comparison of the Dutch and German guidelines on multidrug-resistant Gram-negative microorganisms. Antimicrob Resist Infect Control 2015, 4:7-015-0047-6. eCollection 2015.
2. Woerther PL, Burdet C, Chachaty E, Andremont A: Trends in human fecal carriage of extended-spectrum beta-lactamases in the community: toward the globalization of CTX-M. Clin Microbiol Rev 2013, 26(4):744-758.
3. Arias CA, Murray BE: The rise of the Enterococcus: beyond vancomycin resistance. Nat Rev Microbiol 2012, 10(4):266-278.
4. Lebreton F, van Schaik W, McGuire AM, Godfrey P, Griggs A, Mazumdar V, Corander J, Cheng L, Saif S, Young S, Zeng Q, Wortman J, Birren B, Willems RJ, Earl AM, Gilmore MS: Emergence of epidemic multidrug-resistant Enterococcus faecium from animal and commensal strains. MBio 2013, 4(4):10.1128/mBio.00534-13.
5. Galloway-Pena J, Roh JH, Latorre M, Qin X, Murray BE: Genomic and SNP Analyses Demonstrate a Distant Separation of the Hospital and Community-Associated Clades of Enterococcus faecium. PLoS One 2012, 7(1):e30187.
6. Https://www.Deutschland-Nederland.Eu.
7. Kwong JC, McCallum N, Sintchenko V, Howden BP: Whole genome sequencing in clinical and public health microbiology. Pathology 2015, 47(3):199-210.
8. Leopold SR, Goering RV, Witten A, Harmsen D, Mellmann A: Bacterial whole-genome sequencing revisited: portable, scalable, and standardized analysis for typing and detection of virulence and antibiotic resistance genes. J Clin Microbiol 2014, 52(7):2365-2370.
9. Maiden MC, Jansen van Rensburg MJ, Bray JE, Earle SG, Ford SA, Jolley KA, McCarthy ND: MLST revisited: the gene-by-gene approach to bacterial genomics. Nat Rev Microbiol 2013, 11(10):728-736.
10. de Been M, Pinholt M, Top J, Bletz S, Mellmann A, van Schaik W, Brouwer E, Rogers M, Kraat Y, Bonten M, Corander J, Westh H, Harmsen D, Willems RJ: A core genome MLST scheme for high-resolution typing of Enterococcus faecium. J Clin Microbiol 2015, .
11. Bruijnesteijn van Coppenraet LE, Dullaert-de Boer M, Ruijs GJ, van der Reijden WA, van der Zanden AG, Weel JF, Schuurs TA: Case-control comparison of bacterial and protozoan microorganisms associated with gastroenteritis: application of molecular detection. Clin Microbiol Infect 2015, 21(6):592.e9-592.e19.
12. Werner G, Fleige C, Geringer U, van Schaik W, Klare I, Witte W: IS element IS16 as a molecular screening tool to identify hospital-associated strains of Enterococcus faecium. BMC Infect Dis 2011, 11:80.
13. Clark NC, Cooksey RC, Hill BC, Swenson JM, Tenover FC: Characterization of glycopeptide-resistant enterococci from U.S. hospitals. Antimicrob Agents Chemother 1993, 37(11):2311-2317.
14. Garcia-Cobos S, Kock R, Mellmann A, Frenzel J, Friedrich AW, Rossen JW: Molecular Typing of Enterobacteriaceae from Pig Holdings in North-Western Germany Reveals Extended- Spectrum and AmpC beta-Lactamases Producing but no Carbapenem Resistant Ones. PLoS One 2015, 10(7):e0134533.
15. Http://www.Ridom.de/seqsphere/ug/v20/MLST+_Target_Definer.Html
16. Kluytmans-van den Bergh MF, Rossen JW, Bruijning-Verhagen PC, Bonten MJ, Friedrich AW, Vandenbroucke-Grauls CM, Willems RJ, Kluytmans JA: Whole-Genome Multilocus Sequence Typing of Extended-Spectrum-Beta-Lactamase-Producing Enterobacteriaceae. J Clin Microbiol 2016, 54(12):2919-2927.
17. Wirth T, Falush D, Lan R, Colles F, Mensa P, Wieler LH, Karch H, Reeves PR, Maiden MC, Ochman H, Achtman M: Sex and virulence in Escherichia coli: an evolutionary perspective. Mol Microbiol 2006, 60(5):1136-1151.

18. Https://enterobase.Warwick.Ac.Uk.

19. Clermont O, Bonacorsi S, Bingen E: Rapid and simple determination of the Escherichia coli phylogenetic group. Appl Environ Microbiol 2000, 66(10):4555-4558.

20. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV: Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 2012, 67(11):2640-2644.

21. Carattoli A, Zankari E, Garcia-Fernandez A, Voldby Larsen M, Lund O, Villa L, Moller Aarestrup F, Hasman H: In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother 2014, 58(7):3895-3903.

22. Joensen KG, Scheutz F, Lund O, Hasman H, Kaas RS, Nielsen EM, Aarestrup FM: Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic Escherichia coli. J Clin Microbiol 2014, 52(5):1501-1510.

23. Joensen KG, Tetzschner AM, Iguchi A, Aarestrup FM, Scheutz F: Rapid and Easy In Silico Serotyping of Escherichia coli Isolates by Use of Whole-Genome Sequencing Data. J Clin Microbiol 2015, 53(8):2410-2426.

24. Larsen MV, Cosentino S, Lukjancenko O, Saputra D, Rasmussen S, Hasman H, Sicheritz-Ponten T, Aarestrup FM, Ussery DW, Lund O: Benchmarking of methods for genomic taxonomy. J Clin Microbiol 2014, 52(5):1529-1539.

25. Hasman H, Saputra D, Sicheritz-Ponten T, Lund O, Svendsen CA, Frimodt-Moller N, Aarestrup FM: Rapid whole-genome sequencing for detection and characterization of microorganisms directly from clinical samples. J Clin Microbiol 2014, 52(1):139-146.

26. Overdevest IT, Heck M, van der Zwaluw K, Huijsdens X, van Santen M, Rijnsburger M, Eustace A, Xu L, Hawkey P, Savelkoul P, Vandenbroucke-Grauls C, Willemsen I, van der Ven J, Verhulst C, Kluytmans JA: Extended-spectrum beta-lactamase producing Klebsiella spp. in chicken meat and humans: a comparison of typing methods. Clin Microbiol Infect 2014, 20(3):251-255.

27. Pietsch M, Eller C, Wendt C, Holfelder M, Falgenhauer L, Fruth A, Grossl T, Leistner R, Valenza G, Werner G, Pfeifer Y, RESET Study Group: Molecular characterisation of extended-spectrum beta-lactamase (ESBL)-producing Escherichia coli isolates from hospital and ambulatory patients in Germany. Vet Microbiol 2015, .

28. Reuland EA, Overdevest IT, Al Naiemi N, Kalpoe JS, Rijnsburger MC, Raadsen SA, Ligtenberg-Burgman I, van der Zwaluw KW, Heck M, Savelkoul PH, Kluytmans JA, Vandenbroucke-Grauls CM: High prevalence of ESBL-producing Enterobacteriaceae carriage in Dutch community patients with gastrointestinal complaints. Clin Microbiol Infect 2013, 19(6):542-549.

29. van Hoek AH, Schouls L, van Santen MG, Florijn A, de Greeff SC, van Duijkeren E: Molecular characteristics of extended-spectrum cephalosporin-resistant enterobacteriaceae from humans in the community. PLoS One 2015, 10(6):e0129085.

30. Bar-Yoseph H, Hussein K, Braun E, Paul M: Natural history and decolonization strategies for ESBL/carbapenem-resistant Enterobacteriaceae carriage: systematic review and meta-analysis. J Antimicrob Chemother 2016, 71(10):2729-2739.

31. Ewers C, Bethe A, Semmler T, Guenther S, Wieler LH: Extended-spectrum beta-lactamase-producing and AmpC-producing Escherichia coli from livestock and companion animals, and their putative impact on public health: a global perspective. Clin Microbiol Infect 2012, 18(7):646-655.

32. Reuland EA, Halaby T, Hays JP, de Jongh DM, Snetselaar HD, van Keulen M, Elders PJ, Savelkoul PH, Vandenbroucke-Grauls CM, Al Naiemi N: Plasmid-mediated AmpC: prevalence in community-acquired isolates in Amsterdam, the Netherlands, and risk factors for carriage. PLoS One 2015, 10(1):e0113033.

33. Overdevest IT, Bergmans AM, Verweij JJ, Vissers J, Bax N, Snelders E, Kluytmans JA: Prevalence of phylogroups and O25/ST131 in susceptible and extended-spectrum beta-lactamase-producing Escherichia coli isolates, the Netherlands. Clin Microbiol Infect 2015, 21(6):570.e1-570.e4.

34. van der Bij AK, Peirano G, Goessens WH, van der Vorm ER, van Westreenen M, Pitout JD: Clinical and molecular characteristics of extended-spectrum-beta-lactamase-producing Escherichia coli causing bacteremia in the Rotterdam Area, Netherlands. Antimicrob Agents Chemother 2011, 55(7):3576-3578.

35. de Regt MJ, van der Wagen LE, Top J, Blok HE, Hopmans TE, Dekker AW, Hene RJ, Siersema PD, Willems RJ, Bonten MJ: High acquisition and environmental contamination rates of CC17 ampicillin-resistant Enterococcus faecium in a Dutch hospital. J Antimicrob Chemother 2008, 62(6):1401-1406.

36. Zhou X, Arends JP, Span LF, Friedrich AW: Algorithm for pre-emptive glycopeptide treatment in patients with haematologic malignancies and an Enterococcus faecium bloodstream infection. Antimicrob Resist Infect Control 2013, 2(1):24.

37. Torell E, Cars O, Olsson-Liljequist B, Hoffman BM, Lindback J, Burman LG: Near absence of vancomycin-resistant enterococci but high carriage rates of quinolone-resistant ampicillin-resistant enterococci among hospitalized patients and nonhospitalized individuals in Sweden. J Clin Microbiol 1999, 37(11):3509-3513.

38. Rinke van den Brink: Het einde van de antibiotica. Hoe bacteriën winnen van een wondermiddel. De Geus BV; 2013.

39. van den Braak N, Ott A, van Belkum A, Kluytmans JA, Koeleman JG, Spanjaard L, Voss A, Weersink AJ, Vandenbroucke-Grauls CM, Buiting AG, Verbrugh HA, Endtz HP: Prevalence and determinants of fecal colonization with vancomycin-resistant Enterococcus in hospitalized patients in The Netherlands. Infect Control Hosp Epidemiol 2000, 21(8):520-524.

40. Endtz HP, van den Braak N, van Belkum A, Kluytmans JA, Koeleman JG, Spanjaard L, Voss A, Weersink AJ, Vandenbroucke-Grauls CM, Buiting AG, van Duin A, Verbrugh HA: Fecal carriage of vancomycin-resistant enterococci in hospitalized patients and those living in the community in The Netherlands. J Clin Microbiol 1997, 35(12):3026-3031.

41. Antimicrobial Resistance Interactive Database (EARS-Net) Http://ecdc.Europa.eu/en/

42. Pinholt M, Larner-Svensson H, Littauer P, Moser CE, Pedersen M, Lemming LE, Ejlertsen T, Sondergaard TS, Holzknecht BJ, Justesen US, Dzajic E, Olsen SS, Nielsen JB, Worning P, Hammerum AM, Westh H, Jakobsen L: Multiple hospital outbreaks of vanA Enterococcus faecium in Denmark, 2012-13, investigated by WGS, MLST and PFGE. J Antimicrob Chemother 2015, 70(9):2474-2482.

43. Fischer J, Hille K, Ruddat I, Mellmann A, Kock R, Kreienbrock L: Simultaneous occurrence of MRSA and ESBL-producing Enterobacteriaceae on pig farms and in nasal and stool samples from farmers. Vet Microbiol 2016, .

44. Mellmann A, Bletz S, Boking T, Kipp F, Becker K, Schultes A, Prior K, Harmsen D: Real-Time Genome Sequencing of Resistant Bacteria Provides Precision Infection Control in an Institutional Setting. J Clin Microbiol 2016, 54(12):2874-2881.

Table S1: metrics raw data and assemblies

	Number of contigs <1000	N50 >15.000	Max contig length >50.000	Contig total bp	Coverage >30x	% reads used >90%	% of expected genome size >90% - <115%	Reads count	Reads average length	Count matched
E. coli										
1_Esco_CA-NL	118	191682	358090	5355780	84,94	99,60	98,5	2021636	225,03	2013594
2_Esco_CA-NL	108	163016	364102	5283863	86,38	99,44	97,1	2721752	167,69	2706482
3_Esco_CA-NL	247	99795	322831	5808942	55,29	99,11	106,8	1648839	194,8	1634095
4_Esco_CA-NL	141	98292	267208	5427644	76,43	99,59	99,8	1841350	225,3	1833826
5_Esco_CA-NL	166	76293	203186	4967893	73,15	99,45	91,3	1624869	223,65	1615992
6_Esco_CA-NL	65	193003	705749	4964881	90,86	99,48	91,3	2265390	199,13	2253707
7_Esco_CA-NL	107	125308	478078	4861038	91,40	99,53	89,4	1986025	223,72	1976643
8_Esco_CA-NL	126	135970	505790	5245727	64,62	99,28	96,4	1532958	221,12	1521955
9_Esco_CA-NL	107	126118	355226	4742141	134,39	99,59	87,2	3849660	165,54	3833954
10_Esco_CA-NL	131	68898	215278	4592628	86,85	99,59	84,4	1751629	227,72	1744519
11_Esco_CA-NL	119	148112	373540	5351130	65,41	99,62	98,4	1536467	227,81	1530698
12_Esco_HA-NL	55	223440	572354	4492147	166,69	99,20	82,6	5836391	128,3	5789524
12b_Esco_HA-NL	75	127820	354607	4496772	35,51	99,54	82,7	938899	170,08	934610
13_Esco_HA-NL	86	191392	570686	5038933	100,13	99,34	92,6	2758668	182,9	2740565
14_Esco_HA-NL	175	150214	284655	4832324	93,65	98,84	88,8	3537371	127,93	3496430
15_Esco_HA-NL	138	190832	408059	5347576	97,36	99,39	98,3	3296572	157,93	3276511
16_Esco_HA-NL	119	172411	420618	5194891	84,34	99,55	95,5	3354184	130,63	3339148
17_Esco_HA-NL	116	166159	602705	5399155	127,76	99,50	99,2	4039881	170,74	4019870
18_Esco_HA-NL	137	145507	275025	5215799	52,55	99,22	95,9	1799021	152,35	1784973
19_Esco_HA-NL	105	128899	313660	4866096	111,72	99,59	89,5	3272321	166,14	3258844
20_Esco_HA-NL	132	109269	363528	5099690	84,79	99,34	93,7	2820330	153,31	2801679

	Number of contigs <1000	N50 >15.000	Max contig length >50.000	Contig total bp	Coverage >30x	% reads used >90%	% of expected genome size >90% - <115%	Reads count	Reads average length	Count matched
20b_Esco_HA-NL	86	217811	343117	4857676	41,45	99,58	89,3	1169242	172,21	1164342
21_Esco_HA-NL	136	111742	305200	5279160	75,98	99,57	97,0	1825862	219,69	1818071
22_Esco_HA-NL	89	147942	294513	5142005	86,44	99,62	94,5	2166676	205,15	2158499
22c_Esco_HA-NL	203	62928	186153	5127236	29,91	99,35	94,3	970810	157,98	964475
23_Esco_HA-NL	87	133573	352442	4976051	87,83	99,69	91,5	1900596	229,94	1894797
24_Esco_HA-NL	114	109880	235251	4819129	67,12	99,53	88,6	1411446	229,18	1404817
25_Esco_HA-NL	109	153918	442015	5261580	61,65	98,52	96,7	1463690	221,63	1442025
25b_Esco_HA-NL	458	22197	110084	5244141	20,48	98,84	96,4	656398	163,65	648756
26_Esco_HA-NL	89	174102	404229	5116427	87,50	99,71	94,1	1964945	227,83	1959317
27_Esco_HA-NL	214	65691	247479	5196831	85,17	99,51	95,5	1946210	227,43	1936665
28_Esco_HA-NL	56	173305	457591	4784220	76,49	99,66	87,9	1595930	229,3	1590447
29_Esco_HA-NL	205	89142	203044	5187114	58,84	99,61	95,4	1325362	230,28	1320175
30_Esco_HA-NL	82	148216	451007	4971572	83,66	99,51	91,4	1848393	225,02	1839374
31_Esco_HA-NL	158	83469	233486	5011249	82,42	99,68	92,1	1817965	227,2	1812137
32_Esco_HA-NL	109	153918	442015	5261580	61,65	98,52	96,7	1463690	221,63	1442025
32b_Esco_HA-NL	189	51700	148831	5078255	22,92	99,32	93,4	704857	165,15	700084
33_Esco_HA-NL	58	244539	407504	4995036	117,53	99,66	91,8	3375428	173,92	3364104
33b_Esco_HA-NL	89	253460	414095	4993860	49,76	99,62	91,8	1613400	154,02	1607303
34_Esco_HA-NL	139	112993	295864	5152838	89,12	99,62	94,7	2031654	226,04	2023889
35_Esco_HA-NL	124	119161	359876	5059658	67,81	99,06	93,0	1577416	217,52	1562551
35b_Esco_HA-NL	235	56790	187134	4989725	22,75	99,28	91,7	633934	179,08	629393
36_Esco_HA-NL	132	131356	402574	5095672	71,40	99,52	93,7	2224309	163,57	2213674
37_Esco_HA-DE	108	133196	497892	5370809	84,49	99,70	98,7	1872235	242,36	1866657

	Number of contigs <1000	N50 >15.000	Max contig length >50.000	Contig total bp	Coverage >30x	% reads used >90%	% of expected genome size >90% - <115%	Reads count	Reads average length	Count matched
38_Esco_HA-DE	61	274210	838823	5526501	40,34	99,19	101,6	994920	224,05	986857
39_Esco_HA-DE	44	210315	481462	4692670	76,91	99,64	86,3	1525985	236,52	1520565
40_Esco_HA-DE	114	112692	287503	4908468	42,50	99,45	90,2	861779	242,07	857028
41_Esco_HA-DE	92	140654	439539	4832312	64,40	99,68	88,8	1297992	239,76	1293844
42_Esco_HA-DE	82	274276	718677	4974466	66,48	99,72	91,4	1397607	236,61	1393625
43_Esco_HA-DE	193	70632	152492	4956801	55,29	99,22	91,1	1144482	239,47	1135568
44_Esco_HA-DE	139	112193	287998	5019313	68,54	99,21	92,3	1459096	235,77	1447579
45_Esco_HA-DE	212	66407	203445	5365665	57,30	99,32	98,6	1344500	228,69	1335308
46_Esco_HA-DE	82	218246	358823	4973327	61,95	99,57	91,4	1301009	236,83	1295377
47_Esco_HA-DE	143	119425	279283	5285998	64,06	99,66	97,2	1434793	235,99	1429863
48_Esco_HA-DE	190	75705	319715	5421044	70,00	98,90	99,7	1628210	233,07	1610227
E. faecium										
1_Efcm_CA-NL	99	69218	233412	2528636	101,03	98,97	86,3	1085629	235,31	1074425
2_Efcm_HA-NL	175	36317	148624	2991184	126,05	99,34	102,1	1588025	237,42	1577537
3_Efcm_HA-NL	182	35172	148166	2994909	156,94	99,33	102,2	2019371	232,76	2005812
4_Efcm_HA-NL	178	34655	146554	2941833	130,21	99,44	100,4	1638752	233,75	1629644
5_Efcm_HA-NL	191	31780	111795	2942805	161,66	98,83	100,4	2068528	229,98	2044374
6_Efcm_HA-NL	191	32824	106547	2973627	138,89	98,82	101,5	1770177	233,32	1749347
7_Efcm_HA-NL	142	47512	146738	2884089	143,15	99,60	98,4	1588545	259,89	1582145
8_Efcm_HA-DE	178	34271	134529	2982597	136,10	99,52	101,8	1615214	251,31	1607512
9_Efcm_HA-DE	167	46221	145187	2955402	150,25	99,59	100,9	1756628	252,79	1749495
10_Efcm_HA-DE	221	36608	190812	2987218	169,03	99,58	102,0	1980600	254,94	1972316
11_Efcm_HA-DE	175	37291	134580	3063945	107,29	99,19	104,6	1449009	226,86	1437252

Table S2a: Finished *E.coli* query genomes used in this study to develop and *ad hoc* cgMLST scheme (n=45). One representative isolate of every ST from every collection (community NL (n=10), Dutch hospitals (n=20) and German hospital (n=6) of the present study and 9 *E. coli* genomes from Dutch patients and farmers previously published (de Been et al. 2014)

Strain	Source	Place of isolation	BioSample. No.	Ref.
148	Human (blood)	Utrecht	SAMN02471499	De Been *et al*
320	Human (urine)	Utrecht	SAMN02471480	De Been *et al*
1350	Human (urine)	Leeuwarden	SAMN02471497	De Been *et al*
1365	Human (urine)	Leeuwarden	SAMN02471498	De Been *et al*
597	Human (urine)	Groningen	SAMN02471510	De Been *et al*
606	Human (pulmonary)	Groningen	SAMN02471485	De Been *et al*
FAH1	Human (faeces)	farm A	SAMN02471475	De Been *et al*
FBH1	Human (faeces)	farm B	SAMN02471517	De Been *et al*
FCH1	Human (faeces)	farm	SAMN02471511	De Been *et al*
1_Esco_CA-NL	Human	Community - NL	SAMN05967539	This study
2_Esco_CA-NL	Human	Community - NL	SAMN05977321	This study
3_Esco_CA-NL	Human	Community - NL	SAMN05977322	This study
4_Esco_CA-NL	Human	Community - NL	SAMN05977323	This study
5_Esco_CA-NL	Human	Community - NL	SAMN05977324	This study
6_Esco_CA-NL	Human	Community - NL	SAMN05977325	This study
8_Esco_CA-NL	Human	Community - NL	SAMN05977327	This study
9_Esco_CA-NL	Human	Community - NL	SAMN05977328	This study
10_Esco_CA-NL	Human	Community - NL	SAMN05977329	This study
11_Esco_CA-NL	Human	Community - NL	SAMN05977330	This study
12_Esco_HA-NL	Human	Hospital - NL	SAMN05977331	This study
13_Esco_HA-NL	Human	Hospital - NL	SAMN05977333	This study
14_Esco_HA-NL	Human	Hospital - NL	SAMN05977334	This study
15_Esco_HA-NL	Human	Hospital - NL	SAMN05977335	This study
16_Esco_HA-NL	Human	Hospital - NL	SAMN05977336	This study
17_Esco_HA-NL	Human	Hospital - NL	SAMN05977337	This study
18_Esco_HA-NL	Human	Hospital - NL	SAMN05977338	This study
19_Esco_HA-NL	Human	Hospital - NL	SAMN05977339	This study
20_Esco_HA-NL	Human	Hospital - NL	SAMN05977340	This study
21_Esco_HA-NL	Human	Hospital - NL	SAMN05977342	This study
23_Esco_HA-NL	Human	Hospital - NL	SAMN05977345	This study
24_Esco_HA-NL	Human	Hospital - NL	SAMN05977346	This study
25_Esco_HA-NL	Human	Hospital - NL	SAMN05977347	This study
27_Esco_HA-NL	Human	Hospital - NL	SAMN05977350	This study
28_Esco_HA-NL	Human	Hospital - NL	SAMN05977351	This study
29_Esco_HA-NL	Human	Hospital - NL	SAMN05977352	This study
30_Esco_HA-NL	Human	Hospital - NL	SAMN05977353	This study
32_Esco_HA-NL	Human	Hospital - NL	SAMN05977355	This study
33_Esco_HA-NL	Human	Hospital - NL	SAMN05977357	This study
34_Esco_HA-NL	Human	Hospital - NL	SAMN05977359	This study

Strain	Source	Place of isolation	BioSample. No.	Ref.
37_Esco_HA-DE	Human	Hospital - DE	SAMN05977363	This study
39_Esco_HA-DE	Human	Hospital - DE	SAMN05977365	This study
40_Esco_HA-DE	Human	Hospital - DE	SAMN05977366	This study
41_Esco_HA-DE	Human	Hospital - DE	SAMN05977367	This study
42_Esco_HA-DE	Human	Hospital - DE	SAMN05977368	This study
43_Esco_HA-DE	Human	Hospital - DE	SAMN05977369	This study

de Been, M., V. F. Lanza, M. de Toro, J. Scharringa, W. Dohmen, Y. Du, J. Hu, et al. 2014. Dissemination of cephalosporin resistance genes between escherichia coli strains from farm animals and humans by specific plasmid lineages. PLoS Genetics 10 (12) (Dec 18): e1004776.

Table S2b: Finished *plasmid* genomes for exclusion of genes with BLAST matches >90% and >100bp length found within the query sequences used in this study to develop a cgMLST scheme.

Strain	Plasmid	GenBank Acc. No.
Escherichia coli 042	pAA	NC_017627.1
Escherichia coli APEC O1	pAPEC-O1-R	NC_009838.1
Escherichia coli ETEC H10407	p948	NC_017724.1
Escherichia coli JJ1886	pJJ1886_5	NC_022651.1
Escherichia coli O104:H4 str. 2009EL-2050	p09EL50	NC_018651.1
Escherichia coli O104:H4 str. 2011C-3493	pESBL-EA11	NC_018659.1
Escherichia coli O111:H- str. 11128	pO111_1	NC_013365.1
Escherichia coli O127:H6 str. E2348/69	pE2348-2	NC_011602.1
Escherichia coli O157:H7 EDL933	pO157	NC_007414.1
Escherichia coli O157:H7 str. TW14359	pO157	NC_013010.1
Escherichia coli O157:H7 str. Sakai	pO157	NC_002128.1
Escherichia coli O26:H11 str. 11368	pO26_1	NC_013369.1
Escherichia coli O55:H7 str. CB9615	pO55	NC_013942.1
Escherichia coli O55:H7 str. RM12579	p12579_1	NC_017653.1
Escherichia coli O7:K1 str. CE10	pCE10A	NC_017647.1
Escherichia coli O83:H1 str. NRG 857C	pO83_CORR	NC_017659.1
Escherichia coli PMV-1	pHUSEC411like	NC_022371.1
Escherichia coli SE11	pSE11-1	NC_011419.1
Escherichia coli SE15	pECSF1	NC_013655.1
Escherichia coli UM146	pUM146	NC_017630.1
Escherichia coli UMN026	p1ESCUM	NC_011749.1
Escherichia coli UMNK88	pUMNK88	NC_017645.1
Escherichia coli UTI89	pUTI89	NC_007941.1
Escherichia coli W	pRK1	NC_017637.1
Escherichia coli W	pRK1	NC_017665.1
Escherichia coli Xuzhou21	pO157	NC_017907.1

Table S3: *E. coli* cgMLST 1771 targets.

Available online: https://www.frontiersin.org/articles/10.3389/fmicb.2017.01914/full#supplementary-material

Table S4: Accessory genes included in the wgMLST scheme of *E. coli.*

Available online: https://www.frontiersin.org/articles/10.3389/fmicb.2017.01914/full#supplementary-material

Table S5: E. coli cgMLST allele types for distance calculation and percentage of good targets/genes.

Available online: https://www.frontiersin.org/articles/10.3389/fmicb.2017.01914/full#supplementary-material

Table S6*: E. faecium* cgMLST allele types for distance calculation and percentage of good targets/genes.

Available online: https://www.frontiersin.org/articles/10.3389/fmicb.2017.01914/full#supplementary-material

Table S7: Genetic distance for pairwise comparisons of grouped ESBL- *E. coli* isolates.

						genetic distance	
Sample ID	**ST**	**Phylogroup**	**Origin**	**Ward**	**Groups**	**cgMLST**	**wgMLST**
33_Esco_HA-NL	69	D	HA-NL	Vascular surgery	group 1	0,0006	0,0008
33b_Esco_HA-NL	69	D	HA-NL	Vascular surgery			
7_Esco_CA-NL	10	A	CA-NL	-	group 2	0,0124	0,0135
46_Esco_HA-DE	10	A	HA-DE	ICU			
1_Esco_CA-NL	131	B2	CA-NL	-	group 3	0,0122	0,0104
32b_Esco_HA-NL	131	B2	HA-NL	Vascular surgery			
12_Esco_HA-NL	5463	D	HA-NL	Gynaecology	group 4	0	0,0004
12b_Esco_HA-NL	5463	D	HA-NL	Gynaecology			
22_Esco_HA-NL	38	B1	HA-NL	Dialysis outpatient	group 5a	0,0006	0,0008
22c_Esco_HA-NL	38	D	HA-NL	Dialysis outpatient	group 5a /5b		
38_Esco_HA-DE	38	D	HA-DE		group 5b	0,0063	0,0076
35b_Esco_HA-NL	131	B2	HA-NL	Dialysis outpatient	group 6a	0,0012	0,0009
35_Esco_HA-NL	131	B2	HA-NL	Dialysis outpatient	group 6a / 6b / 6c		
13_Esco_HA-NL	131	B2	HA-NL	Neurology	group 6b	0,0199	0,0208
26_Esco_HA-NL	131	B2	HA-NL	Gynaecology	group 6c	0,0165	0,0170
25_Esco_HA-NL	95	B2	HA-NL	Neurology	group 7	0,0030	0,0046
25b_Esco_HA-NL	95	B2	HA-NL	Neurology			

HA: hospital acquired; CA: community acquired; NL: The Netherlands; DE: Germany

Table S8: Results of ResFinder, VirulenceFinder, PlasmidFinder, and SerotypeFinder for *E. coli* and *E. faecium* isolates.

Available online: https://www.frontiersin.org/articles/10.3389/fmicb.2017.01914/full#supplementary-material

Figure S1: Minimum spanning tree of ESBL-*E. coli* isolates from hospitals and the community. Distance based on a wgMLST of 4100 genes (cgMLST of 1771 genes and 2329 accessory genes) using the parameters "pairwise ignoring missing values" during calculation. Each circle represents a genotype, colors indicate geographical origin and community or hospital. Orange: hospital-The Netherlands; blue: hospital-Germany; green: community-The Netherlands. Number of different alleles are indicated on the edges between connected isolates (nodes). The same groups considered by cgMLST analysis are highlighted. Isolates are presented by their ID and ST.