Escherichia coli Sequence Type 410 Is Causing New International High-Risk Clones

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ABSTRACT *Escherichia coli* sequence type 410 (ST410) has been reported worldwide as an extraintestinal pathogen associated with resistance to fluoroquinolones, third-generation cephalosporins, and carbapenems. In the present study, we investigated national epidemiology of ST410 *E. coli* isolates from Danish patients. Furthermore, *E. coli* ST410 was investigated in a global context to provide further insight into the acquisition of the carbapenemase genes $bla_{OXA-181}$ and bla_{NDM-5} of this successful lineage. From 127 whole-genome-sequenced isolates, we reconstructed an evolutionary framework of *E. coli* ST410 which portrays the antimicrobial-resistant clades B2/H24R, B3/H24Rx, and B4/H24RxC. The B2/H24R and B3/H24Rx clades emerged around 1987, concurrently with the C1/H30R and C2/H30Rx clades in *E. coli* ST131. B3/H24Rx appears to have evolved by the acquisition of the extended-spectrum β-lactamase (ESBL)-encoding gene $bla_{CTX-M-15}$ and an IncFII plasmid, encoding IncFIA and IncFIB. Around 2003, the carbapenem-resistant clade B4/H24RxC emerged when ST410 acquired an IncX3 plasmid carrying a $bla_{OXA-181}$ carbapenemase gene. Around 2014, the clade B4/H24RxC acquired a second carbapenemase gene, bla_{NDM-5}, on a conserved IncFII plasmid. From an epidemiological investigation of 49 *E. coli* ST410 isolates from Danish patients, we identified five possible regional outbreaks, of which one outbreak involved nine patients with $bla_{OXA-181}$- and bla_{NDM-5}-carrying B4/H24RxC isolates. The accumulated multidrug resistance in *E. coli* ST410 over the past two decades, together with its proven potential of transmission between patients, poses a high risk in clinical settings, and thus, *E. coli* ST410 should

be considered a lineage with emerging "high-risk" clones, which should be monitored closely in the future.

IMPORTANCE Extraintestinal pathogenic *Escherichia coli* (ExPEC) is the main cause of urinary tract infections and septicemia. Significant attention has been given to the ExPEC sequence type ST131, which has been categorized as a "high-risk" clone. High-risk clones are globally distributed clones associated with various antimicrobial resistance determinants, ease of transmission, persistence in hosts, and effective transmission between hosts. The high-risk clones have enhanced pathogenicity and cause severe and/or recurrent infections. We show that clones of the *E. coli* ST410 lineage persist and/or cause recurrent infections in humans, including bloodstream infections. We found evidence of ST410 being a highly resistant globally distributed lineage, capable of patient-to-patient transmission causing hospital outbreaks. Our analysis suggests that the ST410 lineage should be classified with the potential to cause new high-risk clones. Thus, with the clonal expansion over the past decades and increased antimicrobial resistance to last-resort treatment options, ST410 needs to be monitored prospectively.

KEYWORDS BEAST, epidemiology, *Escherichia coli*, outbreak, evolution, high-risk clone

*E*scherichia coli sequence type 131 (ST131) is a high-risk clone, defined as (i) globally distributed, (ii) associated with multiple antimicrobial resistance determinants, (iii) able to colonize and persist in hosts for more than 6 months, (iv) capable of effective transmission between hosts, (v) having enhanced pathogenicity and fitness, and (vi) able to cause severe and/or recurrent infections (1). ST131 is associated with a steady increase in antimicrobial resistance globally, including fluoroquinolone, third-generation cephalosporin, and carbapenem resistance (2–4). A recent study of 10 extended-spectrum β-lactamase (ESBL)-producing *E. coli* ST410 isolates from Germany provided preliminary evidence that this lineage includes a new successful clone with cross-sectorial transmission between wildlife, humans, companion animals, and the environment (5, 6). This high-risk potential is supported by two recent reports from China (7) and Italy (8) of ST410 isolates carrying the acquired carbapenemase gene $bla_{OXA-181}$. Similarly, ST410 carrying $bla_{OXA-181}$ has also been shown to be involved in a small hospital outbreak in Denmark (9). Recent complete-genome sequencing of an ST410 isolate from a Danish patient further revealed a multidrug-resistant strain with two carbapenemase genes ($bla_{OXA-181}$ and bla_{NDM-5}) carried on IncX3 and IncF plasmids, respectively (10). Several studies on molecular characterization of carbapenemase-producing *Enterobacteriaceae* (CPE) among inpatients have indicated clonal spread of CPE from patient to patient (11, 12). Carbapenemase genes are often located on mobile elements containing multiple resistance genes (13, 14), leading to potential horizontal transfer to other bacterial species. The mortality associated with invasive infections caused by CPE is high (15), making the spread of CPE an immense clinical concern. As ST410 has been increasingly reported worldwide, it is important to determine whether the ST410 lineage is causing new pandemic high-risk clones similar to ST131.

In the current study, we investigated the epidemiology of third-generation cephalosporin- and carbapenem-resistant ST410 *E. coli* isolates from Danish patients, to elucidate whether multidrug-resistant ST410 was causing national outbreaks or, alternatively, if a global clone was being introduced multiple times. We further aimed to set ST410 into a global context by the addition of genome data from other national collections as well as from publicly available genomes extracted from the EnteroBase (http://enterobase.warwick.ac.uk) database to provide further insight into the acquisitions of the carbapenemase genes $bla_{OXA-181}$ and bla_{NDM-5} of this successful lineage.

(The data were partly presented at DANMAP 2014, DANMAP 2015, and DANMAP 2016.)

TABLE 1 Combination of extended-spectrum β-lactamase, pAmpC, and carbapenemase enzymes in the 127 *Escherichia coli* ST410 genomes

Phenotype	Gene	No. of genomes	% of genomes
None	No gene detected	13	10
ESBL	$bla_{CTX-M-15}$	21	17
	$bla_{CTX-M-1}$	2	2
	bla_{SHV-12}	1	<1
	$bla_{CTX-M-1}/bla_{CTX-M-15}$	1	<1
pAmpC	bla_{CMY-42}	7	6
	bla_{CMY-2}	1	<1
Carbapenemase	$bla_{OXA-181}$	1	<1
ESBL/pAmpC	$bla_{CTX-M-15}/bla_{CMY-2}$	1	<1
	$bla_{CTX-M-15}/bla_{CMY-42}/bla_{DHA-1}$	1	<1
ESBL/carbapenemase	$bla_{CTX-M-15}/bla_{OXA-181}$	3	2
	$bla_{CTX-M-15}/bla_{KPC-2}$	2	2
	$bla_{CTX-M-15}/bla_{NDM-1}$	1	<1
	$bla_{CTX-M-15}/bla_{KPC-3}$	2	2
	$bla_{CTX-M-15}/bla_{OXA-48}$	1	<1
	bla_{TEM-30}/bla_{KPC-3}	2	2
pAmpC/carbapenemase	$bla_{CMY-2}/bla_{OXA-181}$	11	9
	bla_{CMY-2}/bla_{NDM-5}	1	<1
	$bla_{CMY-42}/bla_{OXA-181}$	1	<1
	bla_{DHA-1}/bla_{OXA-48}	1	<1
	$bla_{CMY-2}/bla_{NDM-5}/bla_{OXA-181}$	2	2
ESBL/pAmpC/carbapenemase	$bla_{CTX-M-15}/bla_{CMY-2}/bla_{OXA-181}$	29	23
	$bla_{CTX-M-15}/bla_{CMY-42}/bla_{OXA-181}$	3	2
	$bla_{CTX-M-15}/bla_{CMY-2}$-truncated$/bla_{OXA-181}$	2	2
	$bla_{CTX-M-15}/bla_{CMY-42}/bla_{OXA-48}$	1	<1
	$bla_{CTX-M-15}/bla_{CMY-2}/bla_{NDM-5}/bla_{OXA-181}$	9	7
	$bla_{CTX-M-15}/bla_{CMY-2}/bla_{NDM-4}/bla_{OXA-181}$	3	2
	$bla_{CTX-M-15}/bla_{SHV-12}/bla_{CMY-2}/bla_{NDM-5}$	1	<1
	$bla_{CTX-M-15}/bla_{SHV-12}/bla_{CMY-2}/bla_{NDM-5}/bla_{OXA-181}$	3	2

RESULTS

Genotypic characterization. Genotypic characterization was applied to the 127 *E. coli* ST410 genomes included in the study (49 genomes, collected from 46 patients from the national Danish surveillance program DANMAP, and 78 international genomes), including whole-genome sequencing (WGS)-based resistance gene profiling, subtyping by *fimH* allelic variation, and identification of plasmid replicons and plasmid multilocus sequence typing (pMLST) subtypes (see Table S1 in the supplemental material).

Of the 127 ST410 genomes, 114 genomes contained at least one of 14 different ESBL genes, plasmid-mediated AmpC (pAmpC) genes, or carbapenemase genes or a truncated pAmpC gene, whereas no gene was detected in 13 genomes (Table 1). The majority of the genomes carried $bla_{CTX-M-15}$ (66%), followed by $bla_{OXA-181}$ (53%), bla_{CMY-2} (48%), bla_{NDM-5} (13%), and bla_{CMY-42} (10%) (Table 1).

From the analysis of the quinolone resistance-determining regions (QRDRs) in the 127 ST410 genomes, 115 of the genomes had S83L and D87N amino acid substitutions in *gyrA*, S80I substitution in *parC*, and S458A substitution in *parE*. None of the 127 genomes had amino acid changes in the QRDR of *gyrB* (Table S1).

Two genomes from the United States (SAMN06645838 and SAMN02442857) and one genome from Denmark (SAMN01885884) belonged to *fimH53* (H53). The remaining 124 genomes belonged to *fimH24* (H24) (Table S1).

Of the 127 ST410 genomes, 66 (52%) had an IncX3 replicon and 125 (98%) had an IncF replicon, with the majority (58/125, 46%) belonging to pMLST F1:A1:B49, while 24
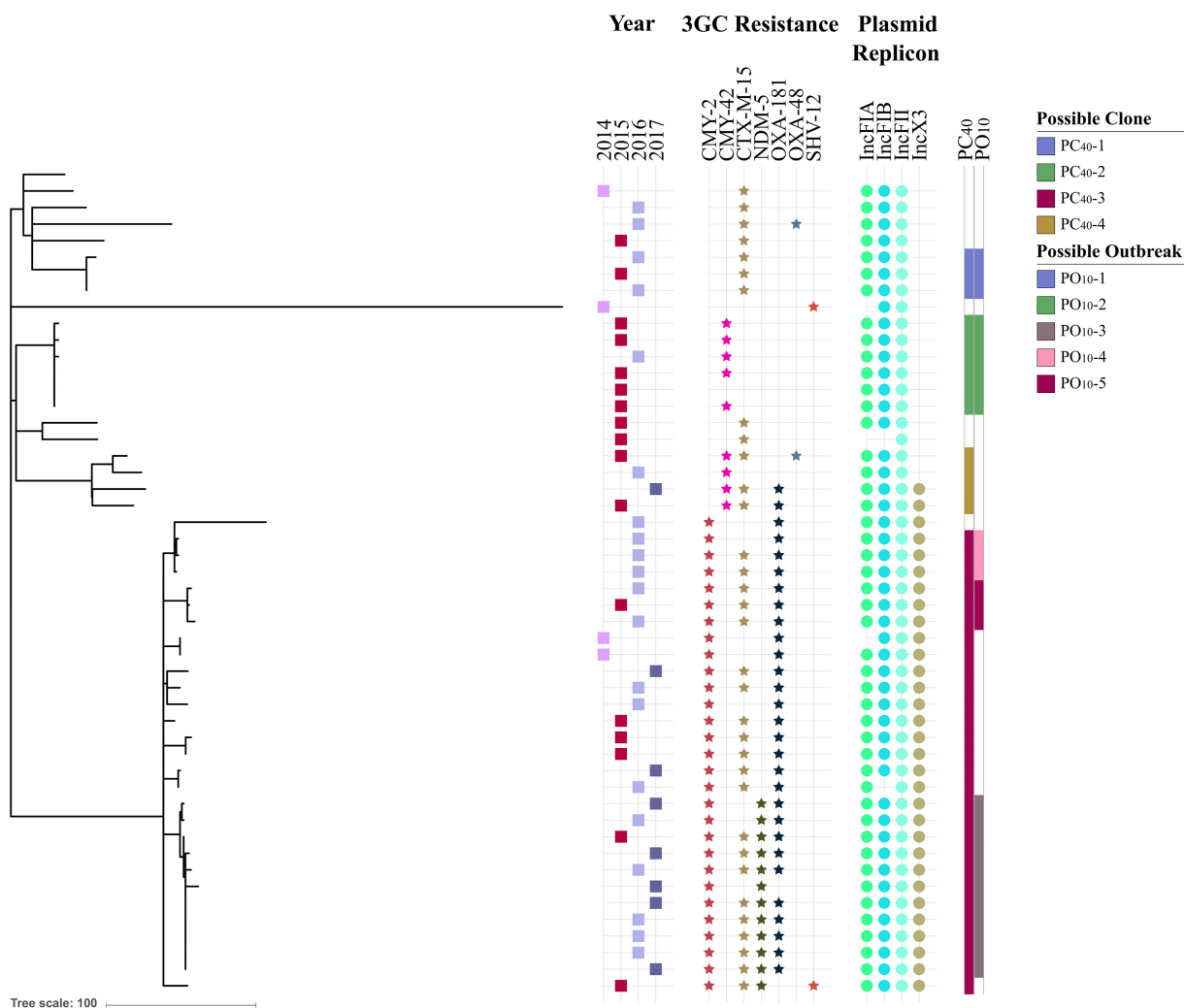
FIG 1 Rooted maximum likelihood phylogeny constructed with Gubbins based on SNP alignment of 49 Danish *E. coli* ST410 genomes. For each isolate, the following metadata are included: year of isolation, associated third-generation cephalosporin resistance (3GC resistance), and presence of IncFIA, IncFIB, IncFII, and IncX3 replicons. Possible clones and outbreaks are colored as per the key.

(19%) belonged to F36:A4:B1, seven (6%) belonged to F31:A4:B1, six (5%) belonged to F48:A1:B49, and three (2%) belonged to F2:A4:B1. For the remaining 27 genomes, 23 different pMLSTs were identified (Table S1).

Epidemiology of *E. coli* ST410 in Denmark. The epidemiology of the 49 ST410 isolates from the Danish surveillance program was investigated by single nucleotide polymorphism (SNP) analysis and visualized with the metadata year of isolation, third-generation cephalosporin resistance (3GC-R) genes, and IncX3 and IncF replicons in a phylogenetic tree (Fig. 1). For construction of the phylogenetic tree, 721 SNPs were identified between the 49 genomes, based on 86% of the reference chromosome after removal of recombinant regions.

The SNP analysis was used to identify possible clones (PC) within the ST410 national Danish strain collection. A pairwise comparison of the SNP differences across the isolates (Fig. S1A) showed a group of isolates with no more than 40 SNPs to the nearest neighbor (0 to 41 SNPs in pairwise comparison), two isolates with 80 SNPs between the genomes, and the remainder with more than 150 SNPs to the nearest neighbor. Adhering to an SNP distance of ≤40 SNPs to the nearest neighboring isolate for describing possible clones (PC_{40}) (Table 2) resulted in four PC_{40}s (PC_{40}-1, -2, -3, and -4). Additionally, possible outbreaks (PO; clusters containing at least three individual isolates) were identified by adhering to an SNP distance of ≤10 SNPs to the nearest

TABLE 2 Possible clones and outbreaks identified in the Danish ST410 *Escherichia coli* collection[a]

PC$_{40}$	No. of genomes in PC$_{40}$	Region(s) (no. of genomes)	No. of SNPs	PO$_{10}$	No. of genomes in PO$_{10}$	Region(s) (no. of genomes)	No. of SNPs	Yr(s) (no. of genomes)	Resistance enzyme(s) (no. of genomes)	Replicon(s)
PC$_{40}$-1	3	Ce (3)	0–4	PO$_{10}$-1	3	Ce (3)	0–4	2015 (1), 2016 (2)	CTX-M-15	IncF
PC$_{40}$-2	6	Ce (6)	0–2	PO$_{10}$-2	6	Ce (6)	0–2	2015 (5), 2016 (1)	CMY-2 (5)	IncF
PC$_{40}$-3	28	Ca (13), Ze (10), So (1), Ce (4)	0–41	PO$_{10}$-3	11	Ca (1), Ze (10)	0–12	2015 (1), 2016 (5), 2017 (5)	CMY-2, OXA-181 (10), NDM-5, CTX-M-15 (8)	IncF, IncX3
				PO$_{10}$-4	3	So (1), Ca (2)	3–6	2016 (3)	CMY-2, OXA-181, CTX-M-15 (2)	IncF, IncX3
				PO$_{10}$-5	3	Ca (1), Ce (2)	2–3	2015 (1), 2016 (2)	CMY-2, OXA-181, CTX-M-15	IncF, IncX3
PC$_{40}$-4	4	Ca (4)	15–38							

[a]Abbreviations: PC$_{40}$, possible clone with ≤40 SNPs to the nearest neighboring isolate; PO$_{10}$, possible outbreak with ≤10 SNPs to the nearest neighboring isolate; Ce, central region of Denmark; Ca, capital region of Denmark; Ze, Zealand region; So, southern region of Denmark.

neighboring isolate (PO$_{10}$). Based on this cutoff value, five possible outbreaks were identified (PO$_{10}$-1, -2, -3, -4, and -5) (Fig. 1).

PC$_{40}$-1 encompassed three genomes with 0 to 4 SNP differences, thus also indicating a possible outbreak (PO$_{10}$-1). The three isolates were from 2015 ($n = 1$) and 2016 ($n = 2$); all three genomes contained the $bla_{CTX-M-15}$ gene and an IncF replicon with pMLST F36:A4:B1. Travel information for the three patients was not available, but the patients had all been hospitalized concurrently in the same hospital unit, R-2 (Fig. S3), in the central region of Denmark (map in Fig. S2).

The second possible clone, PC$_{40}$-2, included six genomes with 0 to 2 SNP differences, also indicating a possible outbreak (PO$_{10}$-2). The six isolates were from 2015 ($n = 5$) and 2016 ($n = 1$) and originated from five patients hospitalized in the central region of Denmark. An IncF replicon with pMLST F48:A1:B49 and bla_{CMY-42} were present in 5/6 genomes. Travel information was unavailable for all five patients associated with these six isolates. However, patients P1, P3, P4, and P5 were hospitalized concurrently at the same hospital unit (R-8) in the beginning of June, and patients P2 and P4 also simultaneously at R-8 in mid-June (Fig. S4).

The third possible clone (PC$_{40}$-3) comprised 28 isolates, collected from patients hospitalized in the capital region of Denmark ($n = 13$), the Zealand region ($n = 10$), the southern region of Denmark ($n = 1$), and the central region of Denmark ($n = 4$). The genomes carried bla_{CMY-2} (28/28) and $bla_{OXA-181}$ (27/28); IncF replicons with pMLST F1:A1:B49 (25/28), F1:A6:B49 (2/28), or F2:A1:B49 (1/28); and an IncX3 replicon (28/28). PC$_{40}$-3 spanned between 0 and 41 SNPs and featured three possible outbreaks, PO$_{10}$-3, PO$_{10}$-4, and PO$_{10}$-5, described in an epidemiological context below.

PO$_{10}$-3 comprised 11 genomes with 0 to 12 SNP differences, collected from nine patients hospitalized during 2015 ($n = 1$), 2016 ($n = 5$), and 2017 ($n = 5$) in the capital region of Denmark ($n = 1$) and the Zealand region ($n = 10$). For two patients, two isolates were included: patient P3 with 11 SNP differences between the isolates collected 13 months apart and patient P6 with three SNP differences between the isolates collected 5 months apart. The genomes in PO$_{10}$-3 carried bla_{NDM-5} (11/11) and $bla_{CTX-M-15}$ (8/11), and all genomes harbored IncF (pMLST F1:A1:B49) and IncX3 replicons. A history of travel to Egypt was registered for P1 from 2015, whereas travel history was unavailable for the remaining 10 patients. Patients P2 to P6 and P8 could be linked through overlapping stays within the same hospital units, whereas patient P7 had been hospitalized at the same hospitals units as P2 to P6 and P8 but 2 or more months apart from the other patients (Fig. S5). No link could be established between patient P1 and the remaining patients. For patient P9, no obvious link could be found to the remaining
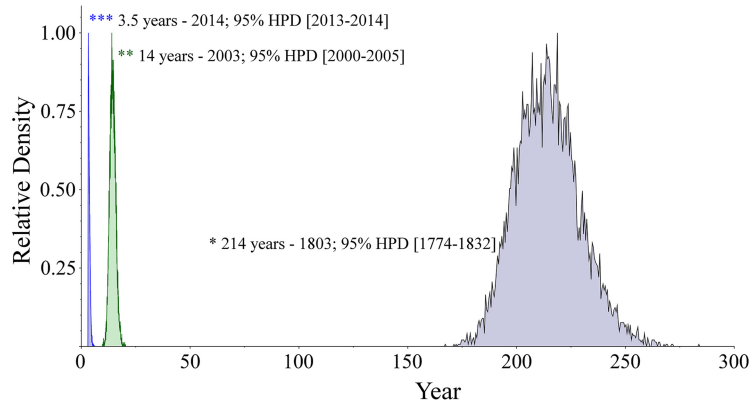
FIG 2 Introduction of OXA-181 and NDM-5 into *E. coli* ST410. Peaks in the density diagram are colored by the introduction of the carbapenemases: green, OXA-181; blue, NDM-5.

patients, but patient P9 was admitted to hospital H shortly after patient P3, though at different units.

PO$_{10}$-4 consists of three isolates with 3 to 6 SNP differences, isolated in 2016 ($n = 3$) in the southern region of Denmark ($n = 1$) and the capital region of Denmark ($n = 2$). The genomes carried $bla_{CTX-M-15}$ (2/3), and all harbored IncF (pMLST F1:A1:B49) and IncX replicons. The three patients did not travel prior to hospitalization. Patients P1 and P3 had been hospitalized 4 months apart in the same hospital unit, but no link could be found to patient P2 (Fig. S6).

PO$_{10}$-5 consisted of three isolates with 2 to 3 SNP differences; they were isolated in 2015 ($n = 1$) and 2016 ($n = 2$), in the capital region of Denmark ($n = 1$) and the central region of Denmark ($n = 2$). The genomes contained $bla_{CTX-M-15}$ (3/3), and all harbored IncF (pMLST F1:A1:B49) and IncX3 replicons. A history of travel to Lebanon was registered for P1 from 2015, while for the remaining two patients, one had no history of recent travel and for the other information on travel history was unavailable. Patients P1 and P3 were admitted at different hospitals, whereas patient P2 was not hospitalized within the investigated time period (Fig. S7).

The fourth possible clone (PC$_{40}$-4) included four isolates with 15 to 38 SNP differences. As the pairwise comparison showed >10 SNPs for all combinations, no regional outbreaks were inferred for this clone. All four isolates carried an IncF replicon with various pMLST profiles (F36:A4:B1, 2/4; F31:A4:B1, 1/4; or F2:A6:B33, 1/4), and two of the isolates carried an IncX3 replicon. The isolates were from 2015 ($n = 2$), 2016 ($n = 1$), and 2017 ($n = 1$) from patients hospitalized in the capital region of Denmark ($n = 4$). Two of the patients had a history of travel to Pakistan, while travel history was unavailable for the remaining two patients.

***E. coli* ST410 in a global context.** The 127 ST410 international isolates spanned more than 4 decades (1975 to 2017) and were from 14 different countries: Denmark ($n = 51$); United Kingdom ($n = 32$); United States ($n = 20$); Germany ($n = 10$); Canada ($n = 4$); Brazil ($n = 2$); and Ireland, Japan, Nepal, Norway, Saudi Arabia, Singapore, Sweden, and Tanzania (one isolate each).

To investigate ST410 in a global context and its temporal dissemination and evolution, a Bayesian coalescent method was applied for phylogenetic reconstruction of the ST410 lineage based on the genomic data and sampling time. By using a general time-reversible (GTR) model and a strict molecular clock with a Bayesian Skyline population model, the BEAST analysis estimated the age of the ST410 lineage to be approximately 214 years (1803; 95% highest posterior density [HPD], 1774 to 1832) (Fig. 2). The phylogenetic reconstruction revealed two distinct clonal lineages of ST410: lineage A with *fimH53* (A/H53) and lineage B with *fimH24* (B/H24) (Fig. 3). The B/H24 lineage was further divided into three sublineages: B2/H24R with the introduction of fluoroquinolone resistance by mutations in *gyrA* and *parC*, B3/H24Rx with the introduction of $bla_{CTX-M-15}$, and B4/H24RxC with the introduction of $bla_{OXA-181}$.

FIG 3 Time-scaled phylogeny of *E. coli* ST410 (*n* = 127) with associated third-generation cephalosporin resistance; plasmid replicons; mutations in the QRDR of *gyrA*, *gyrB*, *parC*, and *parE*; and the possible Danish outbreaks as colored by key. Brackets at right represent ST410 clades as described in the text. The tips of the tree to the left represent the year of isolation for the isolates, with the year stated next to the tips, which is colored by country of isolation, as per the key. ▲, genomes isolated from sources other than human. WT, wild type.

PC$_{40}$-1, PC$_{40}$-2, and PC$_{40}$-4 were all part of the B3/H24Rx complex (Fig. 3). The Danish ST410 isolates in PC$_{40}$-1 and PC$_{40}$-2 from the national analysis did not cluster together with any non-Danish isolates (Fig. 3). The four Danish isolates from PC$_{40}$-4 clustered together with one isolate from the United Kingdom, one isolate from Canada, and two isolates from the United States. For PC$_{40}$-3, an additional 35 isolates clustered together with the 28 Danish isolates, resulting in a distinct clade with 63 isolates (B4/H24RxC in Fig. 3).

PO$_{10}$-4 and PO$_{10}$-5 clustered in separate clades without any additional isolates, whereas PO$_{10}$-3 clustered closely with four isolates from the United Kingdom (7 to 21 SNPs to the nearest neighbor). These 63 isolates were collected between 2013 and 2017, in Denmark ($n = 29$), the United Kingdom ($n = 29$), Canada ($n = 3$), Norway ($n = 1$), and Sweden ($n = 1$). Additionally, 47 of the isolates in B4/H24RxC carried bla$_{OXA-181}$, two isolates carried bla$_{NDM-5}$, and 14 isolates carried both carbapenemases.

Introduction of bla$_{OXA-181}$ and bla$_{NDM-5}$ into ST410. The timing of introductions of bla$_{OXA-181}$ and bla$_{NDM-5}$ into B4/H24RxC was predicted using BEAST (Fig. 3). The introduction of bla$_{OXA-181}$ was estimated to have occurred approximately 14 years ago (2003; 95% HPD, 2000 to 2005), with the subsequent introduction of bla$_{NDM-5}$ 3.5 years ago (2014; 95% HPD, 2013 to 2014).

To investigate if bla$_{OXA-181}$ was carried on an IncX3 plasmid, the genome scaffolds of the 63 ST410 genomes in B4/H24RxC with bla$_{OXA-181}$ and/or IncX3 and an additional six genomes with bla$_{OXA-181}$ and/or IncX3 were compared with the complete plasmid sequence of pAMA1167-OXA-181 from *E. coli* ST410 (10) (Fig. S8A). Overall, the results indicated that the plasmid harboring bla$_{OXA-181}$ is well preserved; only one bla$_{OXA-181}$-positive ST410 isolate, from the United Kingdom, did not carry the IncX3 plasmid (the outermost ring, H14352020701). Additionally, two isolates from Denmark harbored the IncX3 backbone but did not carry the bla$_{OXA-181}$ gene (CPO20150034 and CPO20170049).

To investigate if bla$_{NDM-5}$ was introduced via an IncF plasmid with pMLST F1:A1:B49, the 58 ST410 draft genomes with bla$_{NDM-5}$ and/or IncF with pMLST F1:A1:B49 were compared with the complete plasmid sequence of pAMA1167-NDM-5 (11) (Fig. S8B). All 58 isolates carried genomic information, which covered major parts of the pAMA1167-NDM-5 plasmid backbone, regardless of their bla$_{NDM-5}$ gene status. However, among the 16 bla$_{NDM-5}$-positive genomes, the IncF backbone revealed even higher similarity. The 11 Danish ST410 isolates from PO$_{10}$-3, and four United Kingdom isolates clustering near the PO$_{10}$-3 group, all carried similar IncF backbones (15 innermost rings in Fig. S8B [see also Fig. S9]). However, some of the plasmids lacked various resistance genes, including one United Kingdom isolate (ring 12 from the middle, H15074073205) missing the bla$_{NDM-5}$ gene. Finally, three of the United Kingdom isolates carried bla$_{NDM-4}$ (from the middle; ring 22, H14148045301; 24, H14142058201; and 25, H14140075501) on a plasmid very similar to the bla$_{NDM-5}$ plasmid found in the PO$_{10}$-3 isolates.

DISCUSSION

Recent studies indicate that *E. coli* ST410 is another successful pandemic extraintestinal pathogenic *E. coli* (ExPEC) lineage similar to ST131 (5, 16). Our findings support this hypothesis; however, as national surveillance programs monitor only local epidemiology, global surveillance is required to follow the dissemination of pandemic clones. The limited number of studies on how to interpret phylogenetic relatedness based on SNP differences in *E. coli* makes it difficult to give a generic threshold on how to distinguish clones from local or regional outbreaks when epidemiological data are not available (17–19). Accessing the national collection of *E. coli* ST410 from Denmark, a cutoff value of no more than 40 SNP differences to the nearest neighbor seemed to define clones within this collection, as the closest genome differed by 159 to 185 SNPs to PC$_{40}$-1 to -4 (see Fig. S1B in the supplemental material). The definition of outbreaks being 10 or fewer SNP differences apart from the nearest neighbor has previously been shown plausible (9) and was thus used in this study. Breakdown into possible outbreaks

($\leq$10 SNPs) revealed five possible ST410 outbreaks (PO_{10}s) in Denmark during 2015 to mid-2017. Two of these, PO_{10}-1 and PO_{10}-2, carried $bla_{CTX-M-15}$ and bla_{CMY-42}, respectively. Travel history for all patients in these two PO_{10}s was unavailable, and comparison with the genomes collected from EnteroBase did not reveal any links to other countries. Isolates within these two PO_{10}s were collected from patients within the same region. Epidemiological investigation revealed links between the patients indicating that both could be true outbreaks. This is further supported by the global analysis, where the Danish outbreaks cluster in distinct clades (Fig. 3).

The possible clone PC_{40}-3, including PO_{10}-3, -4, and -5, consisted of 28 isolates with 0 to 41 SNP differences, and most of the isolates carried $bla_{CTX-M-15}$, bla_{CMY-2}, and $bla_{OXA-181}$. Regarding PO_{10}-4 and PO_{10}-5, no epidemiological link could be made between the patients within the possible outbreaks. In PO_{10}-4, the patients did not travel prior to hospitalization, and in PO_{10}-5, one patient had recent travel activity to Lebanon, one patient did not report travel, and travel history was unavailable for the last patient. Thus, it is unclear if multiple introductions occurred, or if unknown carriers or contaminated environmental sources could link the spread between patients from these two PO_{10}s.

Further analysis revealed another possible outbreak (PO_{10}-3) involving 11 isolates from nine patients (P1 to P9), where one patient (P6) contributed with two samples isolated 5 months apart and one patient (P3) contributed with two samples 13 months apart. Isolates from PO_{10}-3 had an additional carbapenemase gene, bla_{NDM-5}. An epidemiological link was found among patients P2 to P8. The patient P9 could possibly be linked to this outbreak via hospital H. We were unable to link patient P1 to the other patients. However, it is not possible to exclude an unknown carrier of the clone, nor an unregistered travel link for patients P2 to P9, whereas patient P1, with the isolate from 2015 (AMA1167), had traveled to Egypt prior to isolation of AMA1167 (10). Also in 2015, an *E. coli* ST410 isolate harboring $bla_{CTX-M-15}$ and bla_{CMY-2} and carrying $bla_{OXA-181}$ on an ~48.5-kb plasmid and bla_{NDM-5} on an ~100-kb plasmid together with FIA and FIB replicons was reported in a study from Egypt (20). The characteristics of this Egyptian OXA-181/NDM-5-producing ST410 isolate correlate with the first OXA-181/NDM-5-producing ST410 isolate collected in Denmark, suggesting that the Danish patient, P1, most likely acquired the isolate during travel to Egypt in 2015. Unfortunately, no WGS data are available for the Egyptian isolate to evaluate such a link.

From the global analysis, the close genetic relationship between four ST410 isolates isolated in the United Kingdom and the genomes from Danish ST410 isolates from PO_{10}-3 could indicate a similar introduction to the United Kingdom; however, two of the patients from the United Kingdom reported no travel prior to hospitalization, and for the remaining two, travel information was unavailable. Similar genetic profiles have previously been reported for NDM-producing *E. coli* ST410 isolates obtained from patients in Denmark and the United Kingdom (21), indicating either similar travel patterns by inhabitants of the two countries or direct transmission between the countries. However, comprehensive epidemiological data with travel information are needed on a global scale to clarify the actual route of transmission.

The coalescence analysis (Fig. 2 and 3) on the global collection of 127 ST410 isolates indicated a most recent common ancestor approximately 214 years ago (the early 1800s), with confidence intervals stretching 30 years backward and forward. In comparison, the time to the most recent common ancestor for the ST131 clade was estimated as the late 1800s (22). Therefore, compared with ST131, ST410 is predicted to be a slightly older lineage of *E. coli*, even though the prevalence of ST131 is far higher than that of ST410.

Several studies have investigated the population structure of ST131 with WGS data and found a sublineage, C/H30, defined by the presence of the *fimH30* allele. Within the C/H30 lineage, the C1/H30R clade, possessing fluoroquinolone resistance conferred by mutations in the chromosomal genes *gyrA* and *parC*, and the C2/H30Rx clade, which in addition is associated with carriage of $bla_{CTX-M-15}$, emerged around 1987 (3, 4, 22, 23). We identified a similar population structure of ST410, with two sublineages based on

the *fimH* alleles: A/H53 (3/127 genomes) and B/H24 (124/127 genomes). In the B/H24 sublineage, the introduction of the fluoroquinolone resistance by mutations in *gyrA* and *parC* (B2/H24R) and the introduction of $bla_{CTX-M-15}$ (B3/H24Rx) were also estimated to have occurred around 1987 concurrently with the widespread clinical introduction of extended-spectrum cephalosporins and fluoroquinolones (Fig. 3). Additionally, the introduction of $bla_{CTX-M-15}$ seems to be related to the acquisition and persistence of an IncFII plasmid harboring FIA/FIB replicons, which was also observed in ST131 (22). In ST410, the introduction of $bla_{OXA-181}$ on an IncX3 plasmid was predicted to have occurred around 2003 (Fig. 3). Furthermore, introduction of bla_{NDM-5}, likely on an IncF replicon, around 3.5 years ago, resulted in a highly resistant clone causing an outbreak (PO_{10}-3) in Denmark.

The majority of the 127 international ST410 isolates in our study were collected from humans (119/127); however, eight isolates were from nonhuman sources. The genomes obtained from isolates from swine ($n = 2$), poultry ($n = 2$), and air from poultry houses ($n = 2$) were all located in the nonresistant clades A/H53 and B1/H24 (Fig. 3), whereas the genomes obtained from sewage and from a dog were both located in the B3/H24Rx cluster. The latter cases likely represent the shared environment with humans. In our study, isolates found in the carbapenemase clade B4/H24RxC were all of human origin. However, carbapenemase genes are often located on mobile genetic elements (13, 14), together with other resistance genes encoding resistance to antibiotic classes commonly used in veterinary medicine (e.g., tetracycline, sulfonamides, or phenicols). Thus, once introduced in animals, carbapenemase genes may be coselected by the use of other classes of antibiotics, which could increase the prevalence of CPE in animals.

CPE is a worrisome risk in public health due to the multidrug resistance and the potential for dissemination to multiple sources, including transmission from patient to patient. The mobile colistin resistance gene *mcr-1* has been reported in ESBL-producing *E. coli* ST410 in Brazil (24) and Germany (25) but was not observed in our 127 isolates. This demonstrates the potential of ST410 to acquire further resistance determinants, leaving very limited therapeutic agents for treating life-threatening bacterial infections.

ST410 *E. coli* represent a globally distributed lineage isolated in Europe, North America, South America, Asia, and Africa and associated with various antimicrobial resistance determinants, including ESBLs, pAmpCs, carbapenemases, and colistin resistance genes. Moreover, our data indicate that ST410 has the ability to persist within a host for long intervals. A subsequent sampling of two patients resulted in the detection of two ST410 isolates in both samples, the first patient with 11 SNP differences between the genomes of isolates collected 13 months apart and the second patient with three SNP differences between the genomes of isolates collected 5 months apart.

The possible outbreaks presented here and previous observations of interspecies transmission of ST410 (5) indicate a potential for effective transmission between patients. Additionally, among the Danish ST410 isolates, 20/49 (41%) were from bloodstream infections, which may qualify for severe infections, and show a potential for enhanced pathogenicity. ST410 belongs to phylogroup A, generally linked to commensal colonization (26), whereas ST131 *E. coli* belongs to phylogroup B2, dominated by strains associated with extraintestinal human infections, adherent-invasive *E. coli* (AIEC), and enteropathogenic *E. coli* (EPEC) (27). Our study demonstrates that parallels can be drawn between the phylogeny and emergence of fluoroquinolone and ESBL resistance (CTX-M-15) in ST131 and ST410. The reduced virulence coupled to phylogroup A could explain why ST410 has not been as globally dominant as ST131, even though ST410 is a slightly older lineage. Further studies are needed to give the complete and true picture of the pathogenicity and fitness of ST410.

These characteristics are properties of an international high-risk clone (1) and underline that ST410 qualifies as a lineage with new international multidrug-resistant high-risk clones, which should be monitored closely in the future.

MATERIALS AND METHODS

Bacterial isolates. Initially, the strain collection at Statens Serum Institut (SSI) containing all ESBL/pAmpC-producing *E. coli* isolates from human bloodstream infections and the carbapenemase-producing organisms (CPO) collected by the Danish Departments of Clinical Microbiology (DCMs) as part of the Danish surveillance program DANMAP between 2014 and mid-2017 were screened for *E. coli* ST410. From the ESBL/pAmpC collection, 14 ST410 isolates from 2014 to 2015 published by Roer et al. (9) and six ST410 isolates from 2016 were obtained (in total, covering 20 isolates from 19 patients). From the CPO surveillance from January 2014 to July 2017, 29 ST410 isolates were included from 27 patients, resulting in a total study population of 49 ST410 isolates from Danish patients. Reported travel information was obtained from the corresponding DCMs as follows: travel-associated and country information (if known), no recent travel activity, or information not available.

Additionally, EnteroBase (https://enterobase.warwick.ac.uk/) was searched for *E. coli* ST410 genomes (accessed 3 July 2017) to extend the study with internationally available ST410 genomes to estimate when the two carbapenemase genes $bla_{OXA-181}$ and bla_{NDM-5} were introduced into the ST410 lineage. Only ST410 genomic data with relevant metadata (year of collection, country, and source type) and availability of raw reads were included. The selection criteria resulted in 43 international *E. coli* genomes: 36 reported to have been collected from humans, two collected from swine, two collected from poultry, two air samples from poultry houses, and one from sewage (see Table S1 in the supplemental material). Finally, sequences from other national surveillance programs with available genome data were included in the study to expand the number and diversity of ST410 genomes harboring $bla_{OXA-181}$ with or without bla_{NDM-5}. Raw reads from 32 genomes were included from the United Kingdom of human origin, one genome from Germany collected from a dog, and one genome each from Norway and from Sweden, both of human origin, resulting in 127 ST410 genomes with relevant metadata and raw reads available for further analysis (Table S1).

Whole-genome sequencing. For isolates that were part of DANMAP surveillance, genomic DNA was extracted (DNeasy Blood and Tissue kit; Qiagen, Copenhagen, Denmark), with subsequent library construction (Nextera kit; Illumina, Little Chesterford, United Kingdom) and finally WGS (MiSeq or NextSeq; Illumina) according to the manufacturer's instructions to obtain paired-end reads of 2 by 250 or 2 by 150 bp in length.

The raw reads of all 127 isolates were assembled into draft genomes using the SPAdes assembler v. 3.10.1 (28).

Genotypic characterization. Draft genomes for the 127 ST410 isolates were submitted to the Center for Genomic Epidemiology (CGE) server through the batch uploader developed for the Bacterial Analysis Platform (BAP) (https://cge.cbs.dtu.dk/services/cge/) (29) and analyzed using the CGE tools described below (analysis performed between 14 August 2017 and 24 August 2017).

(i) Resistance genes and mutations in the QRDRs. Resistance genes were identified with ResFinder 2.1 (30) (included in the CGE BAP), using a threshold of 100% identity (ID) for identifying genes encoding β-lactamases and carbapenemases and 98.00% ID for all other genes encoding transferable antimicrobial resistance. For phenotypically resistant isolates without β-lactamase- and carbapenemase-encoding genes or isolates with less than 100% identity, KmerResistance 1.0 (https://cge.cbs.dtu.dk/services/KmerFinder/) (31) was used to confirm the presence/absence of the gene(s).

To identify mutations in the QRDRs, a nucleotide BLAST database of the 127 ST410 genomes was set up using BLAST 2.6.0+ (32). Gene sequences of *gyrA*, *gyrB*, *parC*, and *parE* were extracted from all genomes by performing a BLASTN query of a representative nucleotide sequence for each of the four genes against this database. The nucleotide sequences were translated into corresponding amino acid sequences based on standard genetic code, using the reading frame beginning from the start codon. Amino acid changes were identified in the QRDRs (GyrA, amino acid position 67 to 106 [33]; GyrB, amino acid position 331 to 480 [34]; ParC, amino acid position 38 to 169 [35]; and ParE, amino acid position 365 to 525 [36]) for each gene by comparison with the wild-type gene variants of the quinolone- and fluoroquinolone-susceptible *E. coli* K-12 MG1655 (GenBank accession no. NC_000913.3).

(ii) Typing of isolates. Multilocus sequence type (MLST) was extracted with MLST 1.6 (37), as part of the CGE BAP, to verify that the genomes belonged to ST410 in the Achtman scheme (MLST 1) (38).

fimH subtypes were identified with FimTyper 1.0 (https://cge.cbs.dtu.dk/services/FimTyper/) (39).

(iii) Plasmid analysis. IncF and IncX3 plasmid replicons were identified using PlasmidFinder 1.4 (40) (included in the CGE BAP) and further subtyped by plasmid MLST (pMLST) with the pMLST 1.4 tool (40).

The genome of the Danish ST410 isolate AMA1167 has previously been completely sequenced (GenBank accession no. GCA_002803905.1) (10), and the complete sequences of the OXA-181 and NDM-5 plasmids were used for comparative plasmid analysis. BLAST analysis of draft genome against pAMA1167-OXA-181 (GenBank accession no. CP024806.1) and pAMA1167-NDM-5 (GenBank accession no. CP024805.1) was performed using the GView server (https://server.gview.ca/).

Genomic epidemiology. (i) Single nucleotide polymorphism analysis. The Northern Arizona SNP Pipeline (NASP) v1.0.0 was used to estimate the molecular epidemiology by identifying SNPs. Briefly, duplicate regions of the reference chromosome of ST410 isolate YD786 (GenBank accession no. NZ_CP013112.1) were identified by aligning the reference against itself with NUCmer v3.1 and masked from downstream analysis. Raw reads of the analyzed genomes were aligned against the FASTA-formatted reference using the Burrows-Wheeler Aligner (BWA), and SNPs were identified using GATK UnifiedGenotyper (41).

Gubbins v2.3.1 was used to remove recombination signals in the SNP output (42). The SNP phylogenies were annotated with relevant metadata using iTOL (http://itol.embl.de) (43).

(ii) Identification of possible clones, outbreaks, and patient links. Possible clones (PC) in the Danish collection were predicted based on the distribution of the pairwise SNP differences between the isolates. Possible clonal outbreaks were identified for clusters containing at least three individual isolates, by adhering to an SNP distance of ≤ 10 SNPs to the nearest neighboring isolate (PO_{10}), as previously described (9).

Patient links in the different possible outbreaks ($PO_{10}s$) were investigated 6 months prior to the first identified isolate in the respective PO_{10} and 6 months after the last identified isolate. Hospital admissions (hospital, unit, and time of admission) were used to construct the timeframe and possible routes of transmission for the $PO_{10}s$.

(iii) Coalescence-based analyses. BEAST analysis was conducted to reconstruct the global phylogeny of ST410 based on the SNP data generated from the 127 raw sequence data sets, for estimation of the time points wherein $bla_{OXA-181}$ and bla_{NDM-5} were acquired by the ST410 lineage. For defining the two groups for $bla_{OXA-181}$ and bla_{NDM-5}, a maximum likelihood phylogeny was estimated with PhyML 3.0 by using the Bayesian information criterion with BIONJ as initial tree, nearest neighbor interchange (NNI) as type of tree improvement, and a bootstrap value of 100 (44). Based on the phylogeny, the two groups were defined.

The timing of the acquisitions of $bla_{OXA-181}$ and bla_{NDM-5} was inferred using coalescence-based analyses in BEAST 1.8.4 at CIPRES Science Gateway (https://www.phylo.org) using duplicate runs. Analyses were performed using different substitution models (general time-reversible [GTR] and Hasegawa-Kishino-Yano [HKY]), strict and relaxed molecular clock, and different demographic models including Bayesian Skyline, constant population, and exponentially growing population tree priors. Chains were sampled every 40,000th step during the 400 million Markov chain Monte Carlo (MCMC) steps. The first 10% of each chain was discarded as burn-in. The best model was selected based on Bayes factor (BF) comparison (45). Data were analyzed with Tracer v1.5 and TreeAnnotator v1.8.4, and the trees were depicted by using iTOL (http://itol.embl.de/) (43).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/mSphere.00337-18.

FIG S1, TIF file, 0.9 MB.
FIG S2, TIF file, 1.1 MB.
FIG S3, TIF file, 0.3 MB.
FIG S4, TIF file, 0.8 MB.
FIG S5, TIF file, 1.5 MB.
FIG S6, TIF file, 0.4 MB.
FIG S7, TIF file, 0.2 MB.
FIG S8, TIF file, 2.6 MB.
FIG S9, TIF file, 2 MB.
TABLE S1, XLSX file, 0.03 MB.

REFERENCES

1. Mathers AJ, Peirano G, Pitout JDD. 2015. The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant Enterobacteriaceae. Clin Microbiol Rev 28:565–591. https://doi.org/10.1128/CMR.00116-14.

2. Peirano G, Bradford PA, Kazmierczak KM, Badal RE, Hackel M, Hoban DJ, Pitout JDD. 2014. Global incidence of carbapenemase-producing Escherichia coli ST131. Emerg Infect Dis 20:1928–1931. https://doi.org/10.3201/eid2011.141388.

3. Price LB, Johnson JR, Aziz M, Clabots C, Johnston B, Tchesnokova V, Nordstrom L, Billig M, Chattopadhyay S, Stegger M, Andersen PS, Pearson T, Riddell K, Rogers P, Scholes D, Kahl B, Keim P, Sokurenko EV. 2013. The epidemic of extended-spectrum-β-lactamase-producing Escherichia coli ST131 is driven by a single highly pathogenic subclone, H30-Rx. mBio 4:e00377-13. https://doi.org/10.1128/mBio.00377-13.

4. Ben Zakour NL, Alsheikh-Hussain AS, Ashcroft MM, Khanh Nhu NT, Roberts LW, Stanton-Cook M, Schembri MA, Beatson SA. 2016. Sequential acquisition of virulence and fluoroquinolone resistance has shaped the evolution of Escherichia coli ST131. mBio 7:e00347-16. https://doi.org/10.1128/mBio.00347-16.

5. Schaufler K, Semmler T, Wieler LH, Wöhrmann M, Baddam R, Ahmed N, Müller K, Kola A, Fruth A, Ewers C, Guenther S. 2016. Clonal spread and interspecies transmission of clinically relevant ESBL-producing Escherichia coli of ST410—another successful pandemic clone? FEMS Microbiol Ecol 92:fiv155. https://doi.org/10.1093/femsec/fiv155.

6. Falgenhauer L, Imirzalioglu C, Ghosh H, Gwozdzinski K, Schmiedel J, Gentil K, Bauerfeind R, Kämpfer P, Seifert H, Michael GB, Schwarz S, Pfeifer Y, Werner G, Pietsch M, Roesler U, Guerra B, Fischer J, Sharp H, Käsbohrer A, Goesmann A, Hille K, Kreienbrock L, Chakraborty T. 2016. Circulation of clonal populations of fluoroquinolone-resistant CTX-M-15-producing Escherichia coli ST410 in humans and animals in Germany. Int

J Antimicrob Agents 47:457–465. https://doi.org/10.1016/j.ijantimicag.2016.03.019.

7. Liu Y, Feng Y, Wu W, Xie Y, Wang X, Zhang X, Chen X, Zong Z. 2015. First report of OXA-181-producing Escherichia coli in China and characterization of the isolate using whole-genome sequencing. Antimicrob Agents Chemother 59:5022–5025. https://doi.org/10.1128/AAC.00442-15.

8. Piazza A, Comandatore F, Romeri F, Pagani C, Floriano AM, Ridolfo A, Antona C, Brilli M, Mattioni Marchetti V, Bandi C, Gismondo MR, Rimoldi SG. 2018. First report of an ST410 OXA-181 and CTX-M-15 coproducing Escherichia coli clone in Italy: a whole-genome sequence characterization. Microb Drug Resist 2017:0366. https://doi.org/10.1089/mdr.2017.0366.

9. Roer L, Hansen F, Thomsen MCF, Knudsen JD, Hansen DS, Wang M, Samulionienė J, Justesen US, Røder BL, Schumacher H, Østergaard C, Andersen LP, Dzajic E, Søndergaard TS, Stegger M, Hammerum AM, Hasman H. 2017. WGS-based surveillance of third-generation cephalosporin-resistant Escherichia coli from bloodstream infections in Denmark. J Antimicrob Chemother 72:1922–1929. https://doi.org/10.1093/jac/dkx092.

10. Overballe-Petersen S, Roer L, Ng K, Hansen F, Justesen US, Andersen LP, Stegger M, Hammerum AM, Hasman H. 2018. Complete nucleotide sequence of an Escherichia coli sequence type 410 strain carrying bla NDM-5 on an IncF multidrug resistance plasmid and bla OXA-181 on an IncX3 plasmid. Genome Announc 6:e01542-17. https://doi.org/10.1128/genomeA.01542-17.

11. Solgi H, Badmasti F, Aminzadeh Z, Giske CG, Pourahmad M, Vaziri F, Havaei SA, Shahcheraghi F. 2017. Molecular characterization of intestinal carriage of carbapenem-resistant Enterobacteriaceae among inpatients at two Iranian university hospitals: first report of co-production of blaNDM-7 and blaOXA-48. Eur J Clin Microbiol Infect Dis 36:2127–2135. https://doi.org/10.1007/s10096-017-3035-3.

12. Ohno Y, Nakamura A, Hashimoto E, Matsutani H, Abe N, Fukuda S, Hisashi K, Komatsu M, Nakamura F. 2017. Molecular epidemiology of carbapenemase-producing Enterobacteriaceae in a primary care hospital in Japan, 2010–2013. J Infect Chemother 23:224–229. https://doi.org/10.1016/j.jiac.2016.12.013.

13. Nordmann P, Poirel L, Walsh TR, Livermore DM. 2011. The emerging NDM carbapenemases. Trends Microbiol 19:588–595. https://doi.org/10.1016/j.tim.2011.09.005.

14. Patel G, Bonomo RA. 2013. 'Stormy waters ahead': global emergence of carbapenemases. Front Microbiol 4:48. https://doi.org/10.3389/fmicb.2013.00048.

15. Mouloudi E, Protonotariou E, Zagorianou A, Iosifidis E, Karapanagiotou A, Giasnetsova T, Tsioka A, Roilides E, Sofianou D, Gritsi-Gerogianni N. 2010. Bloodstream infections caused by metallo-β-lactamase/Klebsiella pneumoniae carbapenemase–producing K. pneumoniae among intensive care unit patients in Greece: risk factors for infection and impact of type of resistance on outcomes. Infect Control Hosp Epidemiol 31:1250–1256. https://doi.org/10.1086/657135.

16. Qin S, Zhou M, Zhang Q, Tao H, Ye Y, Chen H, Xu L, Xu H, Wang P, Feng X. 2016. First identification of NDM-4-producing Escherichia coli ST410 in China. Emerg Microbes Infect 5:e118. https://doi.org/10.1038/emi.2016.117.

17. Lee KI, Morita-Ishihara T, Iyoda S, Ogura Y, Hayashi T, Sekizuka T, Kuroda M, Ohnishi M, EHEC Working Group in Japan. 2017. A geographically widespread outbreak investigation and development of a rapid screening method using whole genome sequences of enterohemorrhagic Escherichia coli O121. Front Microbiol 8:701. https://doi.org/10.3389/fmicb.2017.00701.

18. Joensen KG, Scheutz F, Lund O, Hasman H, Kaas RS, Nielsen EM, Aarestrup FM. 2014. Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic Escherichia coli. J Clin Microbiol 52:1501–1510. https://doi.org/10.1128/JCM.03617-13.

19. Holmes A, Allison L, Ward M, Dallman TJ, Clark R, Fawkes A, Murphy L, Hanson M. 2015. Utility of whole-genome sequencing of Escherichia coli O157 for outbreak detection and epidemiological surveillance. J Clin Microbiol 53:3565–3573. https://doi.org/10.1128/JCM.01066-15.

20. Gamal D, Fernández-Martínez M, El-Defrawy I, Ocampo-Sosa AA, Martínez-Martínez L. 2016. First identification of NDM-5 associated with OXA-181 in Escherichia coli from Egypt. Emerg Microbes Infect 5:e30. https://doi.org/10.1038/emi.2016.24.

21. Nielsen JB, Hansen F, Littauer P, Schønning K, Hammerum AM. 2012. An NDM-1-producing Escherichia coli obtained in Denmark has a genetic pro-

file similar to an NDM-1-producing *E. coli* isolate from the UK. J Antimicrob Chemother 67:2049–2051. https://doi.org/10.1093/jac/dks149.

22. Stoesser N, Sheppard AE, Pankhurst L, De Maio N, Moore CE, Sebra R, Turner P, Anson LW, Kasarskis A, Batty EM, Kos V, Wilson DJ, Phetsouvanh R, Wyllie D, Sokurenko E, Manges AR, Johnson TJ, Price LB, Peto TEA, Johnson JR, Didelot X, Walker AS, Crook DW, Modernizing Medical Microbiology Informatics Group (MMMIG). 2016. Evolutionary history of the global emergence of the Escherichia coli epidemic clone ST131. mBio 7:e02162-15. https://doi.org/10.1128/mBio.02162-15.

23. Petty NK, Ben Zakour NL, Stanton-Cook M, Skippington E, Totsika M, Forde BM, Phan MD, Gomes Moriel D, Peters KM, Davies M, Rogers BA, Dougan G, Rodriguez-Baño J, Pascual A, Pitout JDD, Upton M, Paterson DL, Walsh TR, Schembri MA, Beatson SA. 2014. Global dissemination of a multidrug resistant Escherichia coli clone. Proc Natl Acad Sci U S A 111:5694–5699. https://doi.org/10.1073/pnas.1322678111.

24. Rocha IV, Andrade CADN, Campos TL, Rezende AM, Leal NC, Vidal CFL, Xavier DE. 2017. Ciprofloxacin-resistant and extended-spectrum β-lactamase-producing Escherichia coli ST410 strain carrying the mcr-1 gene associated with bloodstream infection. Int J Antimicrob Agents 49:655–656. https://doi.org/10.1016/j.ijantimicag.2017.03.001.

25. Falgenhauer L, Waezsada SE, Gwozdzinski K, Ghosh H, Doijad S, Bunk B, Spröer C, Imirzalioglu C, Seifert H, Irrgang A, Fischer J, Guerra B, Käsbohrer A, Overmann J, Goesmann A, Chakraborty T. 2016. Chromosomal locations of mcr-1 and blaCTX-M-15 in fluoroquinolone-resistant Escherichia coli ST410. Emerg Infect Dis 22:1689–1691. https://doi.org/10.3201/eid2209.160692.

26. Mushtaq S, Irfan S, Sarma JB, Doumith M, Pike R, Pitout J, Livermore DM, Woodford N. 2011. Phylogenetic diversity of Escherichia coli strains producing NDM-type carbapenemases. J Antimicrob Chemother 66:2002–2005. https://doi.org/10.1093/jac/dkr226.

27. Moriel DG, Tan L, Goh KGK, Phan MD, Ipe DS, Lo AW, Peters KM, Ulett GC, Beatson SA, Schembri MA. 2016. A novel protective vaccine antigen from the core Escherichia coli genome. mSphere 1:e00326-16. https://doi.org/10.1128/mSphere.00326-16.

28. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.

29. Thomsen MCF, Ahrenfeldt J, Cisneros JLB, Jurtz V, Larsen MV, Hasman H, Aarestrup FM, Lund O. 2016. A bacterial analysis platform: an integrated system for analysing bacterial whole genome sequencing data for clinical diagnostics and surveillance. PLoS One 11:e0157718. https://doi.org/10.1371/journal.pone.0157718.

30. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. 2012. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 67:2640–2644. https://doi.org/10.1093/jac/dks261.

31. Clausen PTLC, Zankari E, Aarestrup FM, Lund O. 2016. Benchmarking of methods for identification of antimicrobial resistance genes in bacterial whole genome data. J Antimicrob Chemother 71:2484–2488. https://doi.org/10.1093/jac/dkw184.

32. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. 2009. Blast+: architecture and applications. BMC Bioinformatics 10:421. https://doi.org/10.1186/1471-2105-10-421.

33. Yoshida H, Bogaki M, Nakamura M, Nakamura S. 1990. Quinolone resistance-determining region in the DNA gyrase gyrA gene of Escherichia coli. Antimicrob Agents Chemother 34:1271–1272. https://doi.org/10.1128/AAC.34.6.1271.

34. Jiménez Gómez PA, García de los Rios JE, Rojas Mendoza A, de Pedro Ramonet P, García Albiach R, Reche Sainz MP. 2004. Molecular basis of quinolone resistance in Escherichia coli from wild birds. Can J Vet Res 68:229–231.

35. Vila J, Ruiz J, Goñi P, De Anta MT. 1996. Detection of mutations in parC in quinolone-resistant clinical isolates of Escherichia coli. Antimicrob Agents Chemother 40:491–493.

36. Ruiz J, Casellas S, Jimenez de Anta MT, Vila J. 1997. The region of the parE gene, homologous to the quinolone-resistant determining region of the gyrB gene, is not linked with the acquisition of quinolone resistance in Escherichia coli clinical isolates. J Antimicrob Chemother 39:839–840. https://doi.org/10.1093/jac/39.6.839.

37. Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, Jelsbak L, Sicheritz-Pontén T, Ussery DW, Aarestrup FM, Lund O. 2012.

Multilocus sequence typing of total-genome-sequenced bacteria. J Clin Microbiol 50:1355–1361. https://doi.org/10.1128/JCM.06094-11.

38. Wirth T, Falush D, Lan R, Colles F, Mensa P, Wieler LH, Karch H, Reeves PR, Maiden MCJ, Ochman H, Achtman M. 2006. Sex and virulence in Escherichia coli: an evolutionary perspective. Mol Microbiol 60:1136–1151. https://doi.org/10.1111/j.1365-2958.2006.05172.x.

39. Roer L, Tchesnokova V, Allesøe R, Muradova M, Chattopadhyay S, Ahrenfeldt J, Thomsen MCF, Lund O, Hansen F, Hammerum AM, Sokurenko E, Hasman H. 2017. Development of a web tool for Escherichia coli subtyping based on fimH alleles. J Clin Microbiol 55:2538–2543. https://doi.org/10.1128/JCM.00737-17.

40. Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, Møller Aarestrup F, Hasman H. 2014. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother 58:3895–3903. https://doi.org/10.1128/AAC.02412-14.

41. Sahl JW, Lemmer D, Travis J, Schupp JM, Gillece JD, Aziz M, Driebe EM, Drees KP, Hicks ND, Williamson CHD, Hepp CM, Smith DE, Roe C,

Engelthaler DM, Wagner DM, Keim P. 2016. NASP: an accurate, rapid method for the identification of SNPs in WGS datasets that supports flexible input and output formats. Microb Genomics 2:e000074. https://doi.org/10.1099/mgen.0.000074.

42. Croucher NJ, Page AJ, Connor TR, Delaney AJ, Keane JA, Bentley SD, Parkhill J, Harris SR. 2015. Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins. Nucleic Acids Res 43:e15. https://doi.org/10.1093/nar/gku1196.

43. Letunic I, Bork P. 2016. Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. Nucleic Acids Res 44:W242–W245. https://doi.org/10.1093/nar/gkw290.

44. Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol 59:307–321. https://doi.org/10.1093/sysbio/syq010.

45. Kass RE, Raftery AE. 1995. Bayes factors. J Am Stat Assoc 90:773–795. https://doi.org/10.1080/01621459.1995.10476572.