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Short Communication

First report of the new emerging global clone ST1193 among clinical isolates of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* from Germany

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ABSTRACT

Objectives: Sequence type 1193 (ST1193) is a new emerging global clone of *Escherichia coli*. The main goal of this study was to determine the prevalence and molecular characteristics of ST1193 among clinical isolates of extended-spectrum β-lactamase (ESBL)-producing *E. coli* from University Hospital of Erlangen, Germany.
Methods: Between November 2015 and February 2016, all consecutive non-duplicate clinical *E. coli* isolates showing resistance to cefotaxime or ceftazidime were further analysed for ESBL production by the combined disk method. ESBL genes were identified by PCR and sequencing. Bacterial strain typing was performed by PCR-based phylogrouping, MLST and whole-genome sequencing.
Results: ESBL production was confirmed in 51 isolates. The globally dominant ST131 occurred at a frequency of 37.3% ($n = 19$). Major non-ST131 sequence types were ST38 ($n = 4$; 7.8%), ST10 ($n = 3$; 5.9%) and ST1193 ($n = 3$; 5.9%). Among the ESBL-producing *E. coli* ST1193, two expressed CTX-M-14 and one expressed CTX-M-15 ESBL type. All three ST1193 isolates belonged to serogroup O75:H5, phylogroup B2, and harboured IncFIA and IncFIB plasmids and the virulence factors genes *iha*, *sat*, *gad*, *vat* and *senB*. Moreover, they showed ciprofloxacin resistance and exhibited a set of four conserved mutations defining quinolone resistance (*gyrA* S83L, *gyrA* D87N, *parC* S80I and *parC* L416F).
Conclusions: This study revealed for the first time in Germany the occurrence of ST1193 among clinical isolates of ESBL-producing *E. coli*. Further national or regional multicentre studies are needed to assess the effective relevance of ESBL-producing *E. coli* ST1193 as a nosocomial pathogen in Germany.

1. Introduction

Among clinical isolates of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli*, sequence type 131 (ST131) has emerged worldwide as main agent of urinary tract and bloodstream infections. The successful spread of this clone is associated with several factors, including resistance to fluoroquinolones, affiliation to phylogroup B2, high virulence gene content and possession of the type 1 fimbriae FimH30 allele [1].

Since 2012, a new virulent clone of fluoroquinolone-resistant (FQ^r) *E. coli* belonging to phylogenetic group B2 has been reported in several countries, including Australia, China, Korea, Norway and the USA, namely ST1193 [2–6]. Platell et al. documented the emergence of *E. coli* ST1193 accounting for approximately 10% of a large collection of phylogroup B2 FQ^r clinical isolates of *E. coli* from Australia [4]. In a Korean university hospital, ST1193 has been described as the second most common sequence type (23.7%) after ST131 (37.3%) among FQ^r bloodstream isolates of *E. coli* [3]. In a multicentre surveillance study conducted in the USA, 301 (23.2%) of 1314 FQ^r clinical isolates of *E. coli* belonged to ST1193, 24 (8.0%) of which were ESBL-producers [5]. In a Chinese study, 22 (43.1%) of 51 FQ^r clinical isolates of *E. coli* ST1193 were additionally resistant to cefotaxime and/or ceftazidime and contained ESBL genes. Of these ESBL-positive isolates, 12

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isolates carried $bla_{CTX\text{-}M\text{-}14}$, 6 carried $bla_{CTX\text{-}M\text{-}15}$ and 2 carried $bla_{CTX\text{-}M\text{-}123}$; the remaining 2 isolates co-harboured $bla_{CTX\text{-}M\text{-}14}$ and $bla_{CTX\text{-}M\text{-}15}$ [6]. Moreover, *E. coli* ST1193 has also been described as a commonly occurring variant among FQ^r *E. coli* isolates from faecal samples of healthy individuals in a Norwegian study [2].

To the best of our knowledge, there are no reports regarding the occurrence of ST1193 in Germany. Therefore, the main goal of this study was to determine the prevalence and molecular characteristics of ST1193 among clinical isolates of ESBL-producing *E. coli* from University Hospital of Erlangen (UKER), Erlangen, Germany.

2. Materials and methods

2.1. Study design

UKER is a tertiary-care hospital in Bavaria, Germany, with a capacity of approximately 1400 beds. Between November 2015 and February 2016, all consecutive non-duplicate clinical isolates of *E. coli* from UKER with resistance to cefotaxime [minimum inhibitory concentration (MIC) > 2 mg/L] or ceftazidime (MIC > 4 mg/L) were further analysed for ESBL production and characterisation of ESBL genes. Isolates from rectal swabs or other samples that were exclusively taken to screen for colonisation by ESBL-producing *E. coli* (active surveillance) were not included in this study.

2.2. Definitions

Infections caused by ESBL-producing *E. coli* were recorded according to the criteria published by the German National Reference Center for Surveillance of Nosocomial Infections (NRZ-Hygiene) (http://www.nrz-hygiene.de/en/surveillance/hospital-infection-surveillance-system/kiss-definitionen/). Hospital-acquired infections were defined as infections that occurred >48 h after admission at UKER.

2.3. Bacterial isolates and antimicrobial susceptibility testing

Identification of isolates to species level was performed using matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) (Bruker Daltonik GmbH, Bremen, Germany), and antimicrobial susceptibility testing was performed using a VITEK®2 system (bioMérieux, Marcy-l'Étoile, France). The antimicrobial agents tested were ampicillin, ampicillin/sulbactam, piperacillin, piperacillin/tazobactam, cefuroxime, cefotaxime, ceftazidime, imipenem, meropenem, gentamicin, ciprofloxacin and trimethoprim/sulfamethoxazole (SXT). The results of antimicrobial susceptibility testing were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (http://www.eucast.org/clinical_breakpoints). ESBL or AmpC β-lactamase production was confirmed by the combined disk method (MASTDISCS™ ID AmpC and ESBL Detection Discs; Mast Diagnostica, Reinfeld, Germany) using the following antimicrobial disks: cefpodoxime 10 μg; cefpodoxime 10 μg + ESBL inhibitor; cefpodoxime 10 μg + AmpC inhibitor; and cefpodoxime 10 μg + ESBL inhibitor + AmpC inhibitor.

2.4. Molecular characterisation

Relevant ESBL genes ($bla_{CTX\text{-}M\text{-}1group}$, $bla_{CTX\text{-}M\text{-}2/8group}$, $bla_{CTX\text{-}M\text{-}9group}$, $bla_{SHV\text{-}type}$ and $bla_{TEM\text{-}type}$) and plasmid-mediated quinolone resistance (PMQR) genes [*qnrA/B/C/D/S, qepA and aac(6′)-Ib*-cr] were identified by PCR and sequencing as described previously [7,8]. Phylotyping of all ESBL-producing *E. coli* was performed using a PCR method described by Clemont et al. [9]. Moreover, multilocus sequence typing (MLST) was achieved as recommended by the *E. coli* MLST database (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli) [10]. SeqSphere+ software v.4.0.1 (Ridom GmbH, Münster, Germany) was used for cluster analysis. Minimum spanning tree construction was performed using the parameter 'missing values are an own category'. Selected ESBL-producing *E. coli* were additionally subjected to whole-genome sequencing (WGS) using Illumina MiSeq with 2 × 150-bp paired-end reads. Raw data were processed by SeqSphere+ software v.4.0.1 and assembly was performed using Velvet 1.1.04. Samples were sequenced to aim for a minimum coverage of 120-fold.

WGS data were analysed for serogroup, plasmids, virulence factor genes and mutations defining quinolone resistance using online tools available from the Center for Genomic Epidemiology (CGE) (https://cge.cbs.dtu.dk/services/). Analysis of genetic relatedness was performed by core genome multilocus sequence typing (cgMLST) using SeqSphere+ software v.4.0.1.

3. Results

3.1. Bacterial isolates

In this study, ESBL production was determined by the combined disk method in 51 (94.4%) of 54 clinical *E. coli* isolates showing resistance to third-generation cephalosporins collected from patients at UKER during the period November 2015 to February 2016 (one isolate per patient). The remaining three isolates showed a phenotype that was compatible with the expression of AmpC β-lactamase and therefore were not further analysed.

3.2. Antimicrobial susceptibility

Apart from resistance to third-generation cephalosporins, the 51 ESBL-producing *E. coli* isolates were resistant to SXT ($n = 30$; 58.8%), ciprofloxacin ($n = 29$; 56.9%) and gentamicin ($n = 11$; 21.6%). On the other hand, all of the isolates were susceptible to imipenem and meropenem.

3.3. Clinical epidemiology

The median age of the patients yielding ESBL-producing *E. coli* was 57 years (range 0–87 years). Of the 51 patients, 37 (72.5%) were female and 14 (27.5%) were male. Three patients (5.9%) had a nursing home as their permanent residence. Most of the isolates originated from urine samples ($n = 33$; 64.7%), followed by blood cultures ($n = 6$; 11.8%), wound swabs ($n = 3$; 5.9%) and respiratory material ($n = 3$; 5.9%). Overall, eight isolates (15.7%) originated from the intensive care unit (ICU). According to the criteria of the NRZ-Hygiene, infection occurred in 46 (90.2%) of 51 patients positive for ESBL-producing *E. coli*. The most frequent infections were urinary tract infection ($n = 30$; 65.2%), followed by intra-abdominal infection ($n = 3$; 6.5%), surgical site infection ($n = 3$; 6.5%), pneumonia ($n = 2$; 4.3%) and primary bacteraemia ($n = 2$; 4.3%). Hospital-acquired infections developed in 12 patients (26.1%).

3.4. Molecular characterisation

Fifty ESBL-producing *E. coli* isolates could be assigned by MLST to 23 different sequence types, whereas one isolate could not be assigned (Fig. 1). The globally dominant ST131 occurred with an overall frequency of 37.3% ($n = 19$). Major non-ST131 sequence types were ST38 ($n = 4$; 7.8%), ST10 ($n = 3$; 5.9%) and ST1193 ($n = 3$; 5.9%). The most commonly detected ESBL variants were CTX-M-15 ($n = 21$; 41.2%), CTX-M-1 ($n = 12$; 23.5%), CTX-M-14 ($n = 9$; 17.6%) and CTX-M-7 ($n = 5$; 9.8%). Furthermore, phylogenetic typing showed the predominance of phylogroup B2 ($n = 27$; 52.9%), followed by phylogroup A ($n = 12$; 23.5%), phylogroup D ($n = 7$;

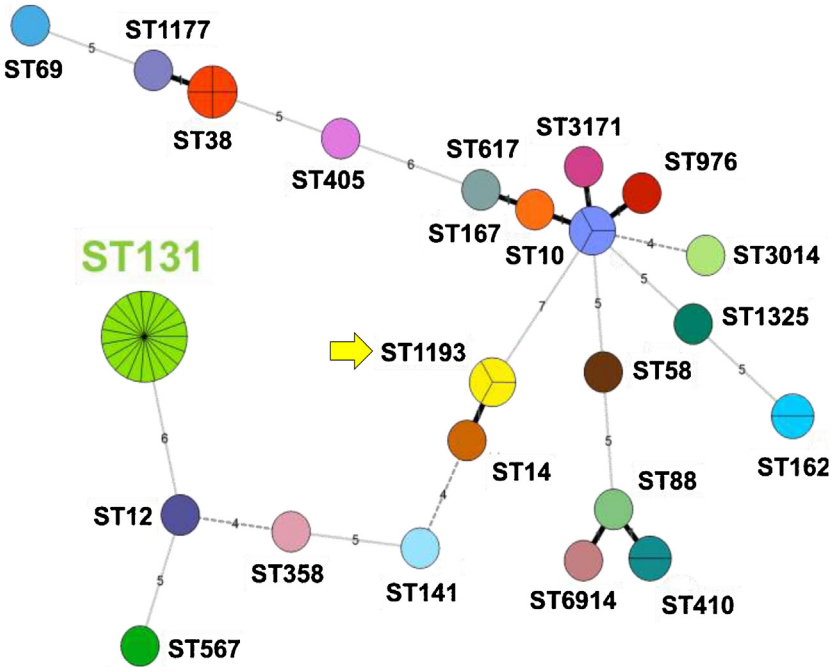

Fig. 1. Minimum spanning tree generated from sequence types (STs) identified among extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolates ($n = 51$) analysed in this study by multilocus sequence typing (MLST). STs are displayed by colour-shaded grouped circles, with the size being proportional to the number of strains. Branch numbers indicate allele differences between STs.

13.7%) and phylogroup B1 ($n = 5$; 9.8%). The distribution of CTX-M types for the most abundant sequence types is shown in Table 1. PMQR genes could be detected in 11 (21.6%) ESBL-producing *E. coli*, of which 9 (81.8%) harboured *aac(6′)-Ib*-cr and the remaining 2 (18.2%) harboured *qnrS*. Isolates showing ST1193 underwent WGS and were subsequently analysed using the Web-based tools of the CGE (see Section 2.4). The most relevant characteristics of the ST1193 isolates in this study are shown in Table 2. Among others, two isolates expressed the CTX-M-14 ESBL type and one isolate expressed the CTX-M-15 ESBL type. All three ST1193 isolates belonged to serogroup O75:H5, phylogroup B2, and harboured IncFIA and IncFIB plasmids and the virulence factors genes *iha* (adherence protein), *sat* (secreted autotransporter toxin), *gad* (glutamate decarboxylase), *vat* (vacuolating autotransporter toxin) and *senB* (plasmid-encoded enterotoxin). Moreover, they showed ciprofloxacin resistance and exhibited a set of four conserved mutations defining quinolone resistance (*gyrA* S83L, *gyrA* D87N, *parC* S80I and *parC* L416F). Analysis of genetic relatedness performed by cgMLST showed a difference between ST1193 isolates of 20 to 34 alleles.

Table 1
Distribution of CTX-M types for the most abundant extended-spectrum β-lactamase-producing *Escherichia coli* sequence types (STs) ($n > 2$).

ST	*N* (%)			
	CTX-M-1	CTX-M-14	CTX-M-15	CTX-M-27
ST131 ($n = 19$)	2 (10.5)	2 (10.5)	10 (52.6)	5 (26.3)
ST38 ($n = 4$)	0	3 (75.0)	1 (25.0)	0
ST10 ($n = 3$)	1 (33.3)	2 (66.7)	0	0
ST1193 ($n = 3$)	0	2 (66.7)	1 (33.3)	0

4. Discussion

In Germany, the rate of clinical *E. coli* isolates showing combined resistance to third-generation cephalosporins and fluoroquinolones has increased during the period 2008–2014 from 5.1% to 8.9% in the hospital setting and from 7.6% to 11.5% in ICUs (https://ars.rki.de/Content/Database/Multiresistance.aspx; accessed 13 October 2018). This finding is primarily associated with the spread of *E. coli* ST131. Data on ESBL-producing *E. coli* ($n = 127$) from different German hospitals during the period 2011–2012 revealed that CTX-M-15 (49.6%) and CTX-M-1 (30.7%) were the most common ESBL types. In addition, ST131 was the most common sequence type with a frequency of 32.3%, and most of these ST131 isolates (87.8%) were additionally resistant to fluoroquinolones [11]. In another German study conducted at University Medical Center Göttingen, CTX-M-1 occurred at a higher frequency than CTX-M-15 (44.4% vs. 34.4%) among 160 ESBL-producing *E. coli* isolates. Moreover, ST131 occurred with a frequency of 24.3%, followed by ST101 (5.0%) and ST58 (5.0%) [12]. Notably, the occurrence of *E. coli* ST1193 was not reported in either of the abovementioned studies.

In this study, 10.3% (51/495) of all *E. coli* isolates from infections analysed during the period November 2015 to February 2016 were ESBL-producers. Moreover, the rate of ESBL-producing *E. coli* ST1193 amounted to 0.6% (3/495). The distribution of ESBL types was in accordance with the data on ESBL-producing *E. coli* from different German hospitals during the period 2011–2012 [11]. To the best of our knowledge, this study revealed for the first time in Germany the occurrence of ST1193 among clinical isolates of ESBL-producing *E. coli*. Although clonally unrelated, all ST1193 isolates in this study were resistant to ciprofloxacin and showed serogroup O75:H5 and phylogroup B2 as previously described [4,6]. Moreover, they harboured IncFIA and IncFIB plasmids. Plasmids belonging to the IncF family are frequently detected in *E. coli* and have already been described as carriers of ESBL genes such as $bla_{\text{CTX-M-14}}$ and $bla_{\text{CTX-M-15}}$ as well as PMQR genes such as *aac(6′)-Ib*-cr, *qnr* and *qepA* [13]. Ciprofloxacin resistance in *E. coli* ST1193 in this study was mainly due to the accumulation of four point mutations in DNA gyrase and topoisomerase IV (*gyrA* S83L, *gyrA* D87N, *parC* S80I and *parC* L416F). Three of these mutations also occurred in ST1193 isolates from Australia and China [4,6]. In addition, the occurrence of an identical virulence gene profile in the ST1193 isolates in this study together

Table 2
Characteristics of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* ST1193 in this study ($n = 3$).

	Isolate 1	Isolate 2	Isolate 3
ID no.	UR08832	UR09651	UR00446
Source	Urine	Urine	Urine
Infection	UTI	UTI	UTI
Serotype	O75:H5	O75:H5	O75:H5
FimH type	64	64	64
ESBL type	CTX-M-14	CTX-M-15	CTX-M-14
Phylogroup	B2	B2	B2
Ciprofloxacin resistance	+	+	+
Chromosomal mutations defining quinolone resistance	*gyrA* S83L, *gyrA* D87N, *parC* S80I, *parC* L416F	*gyrA* S83L, *gyrA* D87N, *parC* S80I, *parC* L416F	*gyrA* S83L, *gyrA* D87N, *parC* S80I, *parC* L416F
PMQR determinants	–	–	–
Plasmids	IncFIA, IncFIB, IncL/M	IncFIA, IncFIB, IncI1, IncQ1	IncFIA, IncFIB
Virulence factor genes[a]	*iha*, *sat*, *gad*, *vat*, *senB*	*iha*, *sat*, *gad*, *vat*, *senB*	*iha*, *sat*, *gad*, *vat*, *senB*

UTI, urinary tract infection; PMQR, plasmid-mediated quinolone resistance.
[a] *iha*, adherence protein; *sat*, secreted autotransporter toxin; *gad*, glutamate decarboxylase; *vat*, vacuolating autotransporter toxin; *senB*, plasmid-encoded enterotoxin.

with the results of serotyping, phylotyping and molecular characterisation of ciprofloxacin resistance confirm that *E. coli* ST1193 strains have a high level of homogeneity independently from the geographic region of origin [4,6].

In conclusion, this study revealed for the first time in Germany the occurrence of ST1193 among clinical isolates of ESBL-producing *E. coli*. Rapid spread of ESBL-producing *E. coli* ST1193 in German hospitals in the ensuing years cannot be excluded, since fluoroquinolones (11.3%) and third-generation cephalosporins (8.9%) are the most commonly used antimicrobials in Germany after penicillins/β-lactamase inhibitors (23.2%) and second-generation cephalosporins (12.9%) [14]. Therefore, further national or regional multicentre studies are needed to assess the effective relevance of ESBL-producing *E. coli* ST1193 as a nosocomial pathogen in Germany.

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Competing interests

None declared.

Ethical approval

Ethical approval was not required as the study was regarded as ordinary surveillance of hospital-acquired infections and multidrug-resistant pathogens at University Hospital of Erlangen (Erlangen, Germany).

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