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SHORT REPORT
Multilocus sequence typing and CTX-M characterization of ESBL-producing *E. coli*: a prospective single-centre study in Lower Saxony, Germany

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SUMMARY

The increasing prevalence of extended-spectrum β-lactamase (ESBL)-producing Gram-negative bacteria is a serious threat for current healthcare settings. In this study we investigated the molecular epidemiology of ESBL-producing *E. coli* at the University Medical Center Göttingen in Lower Saxony, Germany. All *E. coli* isolates with an ESBL phenotype were collected during a 6-month period in 2014. Multilocus sequence typing and CTX-M characterization were performed on 160 isolates. Of the ESBL-producing isolates 95·6% were CTX-M positive. Compared to recent Germany-wide studies, we found CTX-M-1 to occur in higher frequency than CTX-M-15 (44·4% *vs.* 34·4%). CTX-M-14 and CTX-M-27 were detected at 9·4% and 5·0%, respectively. The globally dominant sequence type (ST) 131, which is often associated with CTX-M-15, occurred at a relatively low rate of 24%. Major non-ST131 sequence types were ST101 (5%), ST58 (5%), ST10 (4·4%), ST38 (4·4%), ST410 (3·8%) and ST453 (3·1%). Several of these major sequence types were previously shown to be associated with livestock farming. Together, our study indicates that *E. coli* lineage distribution in individual healthcare settings can significantly differ from average numbers obtained in nationwide studies.

Key words: Antibiotic resistance, *Escherichia coli* (*E. coli*), hospital microbiology, molecular epidemiology.

Escherichia coli isolates from humans and livestock are increasingly resistant to third- and fourth-generation cephalosporins, such as cefotaxime, ceftazidime and cefepime. The major cause of this resistance is the expression of plasmid or chromosomal located genes encoding for either extended-spectrum-β-lactamases (ESBLs) or for AmpC-β-lactamases [1–3]. The frequency of individual ESBL genotypes and their association with certain *E. coli* lineages and clones is undergoing constant change. Over the past 20 years, ESBLs of the genotype CTX-M have emerged as predominant ESBLs in *E. coli* worldwide [4, 5]. From the >100 different CTX-M variants described so far, CTX-M-15 is currently the most frequent genotype identified from ESBL-producing *E. coli* [6, 7]. CTX-M-15 is often associated with sequence type (ST) 131 and serotype O25b, which is the predominant lineage of extraintestinal pathogenic *E. coli* in recent years [8, 9]. In a recent Germany-wide study, the ESBL genotypes and multilocus sequence types of 233 ESBL-producing *E. coli* isolates obtained from German hospitals and medical practices in 2011–2012 were determined [10]. Next to CTX-M-15 (50·4%), the genotypes CTX-M-1 (28·4%) and CTX-M-14 (5·6%) were found to be the most common variants.

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Table 1. *Clinical specimens of the isolates (n = 160)**

Specimen	*N*	ST131†‡ *n* (%)	Non-ST131§‖ *n* (%)
Urine	79	23 (29·1)	56 (70·9)
Rectal swab	14	6 (42·9)	8 (57·1)
Nasopharyngeal swab	10	0 (0)	10 (100)
Wound swab	9	0 (0)	9 (100)
Groin swab	8	3 (37·5)	5 (62·5)
Swab, other locations	13	2 (15·4)	11 (84·6)
Blood culture	6	1 (16·7)	5 (83·3)
Respiratory material	13	3 (23·1)	10 (76·9)
Others	8	1 (12·5)	7 (87·5)

* Median age of all patients: 72 years.
† Median age of patients with ST131 isolates: 71 years.
‡ Nine of 39 ST131 isolates were screening isolates.
§ Median age of patients with non-ST131 isolates: 72 years.
‖ Twenty of 121 non-ST131 isolates were screening isolates.

In this study we characterized ESBL-producing *E. coli* isolates from a single centre, the University Medical Center Göttingen (UMG) in south Lower Saxony, Germany. The UMG is a maximum-care hospital with a capacity of about 1500 beds. Annually around 60 000 patients are admitted and about 173 000 patients are seen in the different outpatient departments.

During November 2013 and May 2014 all *E. coli* isolates from UMG with a cefotaxime resistance >2 mg/l and/or a ceftazidime resistance >4 mg/l were collected. Species identification was performed by MALDI-TOF mass spectroscopy (Bruker, Germany). Antibiotic resistance was determined by VITEK 2 analysis (bioMérieux, France). The collected isolates were adjusted for re-isolates from the same patient, resulting in a total of 160 isolates (Table 1), 79 (49·4%) of which were isolated from urine. The median age of patients was 72 years. Multilocus sequence typing analysis was performed as previously described by amplification and sequencing of fragments from seven housekeeping genes [11]. The sequence type was determined using the University of Warwick database (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli). ST131 occurred with an overall frequency of 24% ($n = 39$) in the collection. Table 1 shows the ST131 proportion for the individual clinical specimens. The specimens with the highest ST131 prevalence were rectal swabs (42·9%, $n = 14$), groin swabs (37·5%, $n = 8$) and urine (29·1%, $n = 79$). The remaining 76% ($n = 121$) belonged to 51 different sequence types. The most prevalent non-ST131 were ST101 (5·0%, $n = 8$), ST58 (5·0%, $n = 8$), ST10 (4·4%, $n = 7$), ST38 (4·4%, $n = 7$), ST410 (3·8%, $n = 6$) and ST453 (3·1%, $n = 5$) (Table 2). A list of all identified sequence types is provided in Supplementary Table S1.

At least four (ST10, ST38, ST58, ST410) out of these six major sequence types have been previously described to be associated with animals and livestock farming [10, 12–15]. Lower Saxony is known as important livestock producing state within Germany [16]. ST38 was also reported to be isolated frequently from healthy humans [17]. Interestingly, the two most abundant non-ST131 sequence types (ST101 and ST58) were identified only at a very low frequency in a Germany-wide surveillance study [10], indicating that significant local differences can occur in sequence-type distribution of ESBL-positive *E. coli*. Outside Germany, CTX-M-positive ST58 were isolated from various animals, e.g. dogs, rooks and poultry [18]. The globally successful ST101 was found to be associated with the metallo-β-lactamase NDM-1, which confers carbapenem resistance, but was also shown to possess less virulence factors than ST131 [19]. ST453 was described as an emerging sequence type associated with extraintestinal infections, particularly urinary tract infections throughout the world [20].

CTX-M genotyping was performed according to Strauss *et al.* [21]. In short, a $bla_{\text{CTX-M}}$ multiplex polymerase chain reaction (PCR) with four primer pairs for $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M-9}}$, and $bla_{\text{CTX-M-8/25}}$ was performed (Supplementary Table S2). PCR products were analysed on a 1·5% agarose gel and purified using the Qiagen PCR purification kit (Qiagen, Germany). After DNA sequencing of the PCR product from both sites (Seqlab, Göttingen), the CTX-M genotype was determined by BLAST-N analysis in the NCBI database. All ST131 isolates ($n = 39$) and 94·3% ($n = 114$) of the non-ST131 isolates were CTX-M positive. The predominant CTX-M variants were CTX-M-1 (44·4%, $n = 71$), CTX-M-15 (34·4%, $n = 55$), CTX-M-14 (9·4%, $n = 15$) and CTX-M-27 (5·0%, $n = 8$) (Fig. 1).

A high prevalence of CTX-M-1, CTX-M-14 and CTX-M-15 is often observed in *E. coli* with the ESBL phenotype and CTX-M-15 is currently the most frequent CTX-M gene in German healthcare settings [2, 10, 22]. The relatively low CTX-M-15 rate of 34·4% in our study appears to be due to the lower ST131 proportion in our collection (24%), compared to a ST131 rate of 35·8% in the Germany-wide study of Pietsch *et al.* [10]. The widely disseminated *E. coli* clone O25b:H4-ST131 frequently carries the CTX-M-15 gene, while non-ST131 sequence types

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Table 2. *CTX-M-type distribution for the most abundant sequence types (n > 3) from ESBL-producing* E. coli

	CTX-M-1	CTX-M-14	CTX-M-15	CTX-M-27
ST131 (*n* = 39)	10·3	12·8	56·4	20·5
ST101 (*n* = 8)	100	0	0	0
ST58 (*n* = 8)	62·5	12·5	25	0
ST10 (*n* = 7)	71·4	0	28·6	0
ST38 (*n* = 7)*	28·6	28·6	28·6	0
ST410 (*n* = 6)*	0	0	83·3	0
ST453 (*n* = 5)	100	0	0	0
ST73 (*n* = 4)*	25	25	0	0
ST648 (*n* = 4)	0	0	100	0
ST744 (*n* = 4)	75	25	0	0

Values given are percentages.
* In addition to the shown CTX-M types, one ST38 isolate was positive for CTX-M-55 and one ST410 isolate was positive for CTX-M-17. Two ST73 isolates were CTX-M negative.

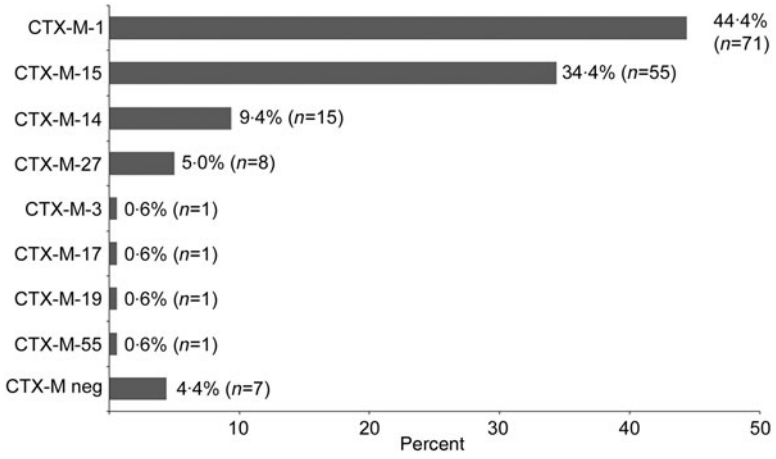

Fig. 1. CTX-M-type distribution in ESBL-producing *E. coli*. Of 160 isolates 153 (95·6%) were CTX-M positive. Eight different CTX-M variants were identified.

show, on average, lower CTX-M-15 associations [8, 9]. However, the proportion of CTX-M-1, CTX-M-15 and CTX-M-14 in our study is similar to the results of a case-control study performed at the Charité University Hospital in Berlin, Germany, with patients colonized with community-acquired ESBL-positive *E. coli* [23].

The individual *E. coli* sequence types in our study displayed significant differences in the distribution of the various CTX-M types (Table 2). The predominant CTX-M variant in ST131 isolates was CTX-M-15 (56·4%, *n* = 22), followed by CTX-M-27 (20·5%, *n* = 8), CTX-M-14 (12·8%, *n* = 5) and CTX-M-1 (10·3%, *n* = 4). In contrast, CTX-M-1 was the predominant genotype in non-ST131 isolates, namely in the abundant sequence types ST101 (8/8 isolates), ST58 (5/8 isolates), ST10 (5/7 isolates) and ST453 (5/5 isolates). An association of ST101, ST453 and ST10 with CTX-M-1 was also reported in a recent Germany-wide study [10]. From the six most abundant sequence types only ST410 shows an association with CTX-M-15 (5/6 isolates). This association of ST410 and CTX-M-15 was also observed in ESBL-positive *E. coli* collected from German and Brazilian hospital patients [10, 24]. Recent data revealed genetic similarities between human and animal CTX-M-15-positive ST410 isolates and suggest a clonal dissemination of specific *E. coli* ST410 clades [25]. The second most common CTX-M variant in our ST131 isolates, i.e. CTX-M-27, was absent in all non-ST131 isolates and was also found at a significantly lower rate (1/127 isolates) in nosocomial patients from other German hospitals [10]. CTX-M-27 differs from CTX-M-14 by a single $Asp^{240}Gly$ substitution that was shown to confer higher ceftazidime resistance (minimum inhibitory concentration: 8 *vs.* 1 mg/l) [26].

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CTX-M-27 is the predominant allele in ST131 isolates from Japanese hospitals. A recent study reported a CTX-M-27 frequency of 45% in ST131 isolates collected at 10 Japanese acute-care centres [27].

In conclusion, our single-centre study in Lower Saxony, Germany reveals a distinct sequence-type distribution of ESBL-producing *E. coli* compared to the average nationwide sequence-type distribution. This leads as a consequence to a shift in the distribution of CTX-M alleles, with CTX-M-1, not CTX-M-15, as the most frequent allele. Local differences in sequence-type frequency might reflect potential area-dependent differences in the proportion of livestock-associated sequence types, e.g. ST10, ST38 and ST410. The greater abundance of these livestock-associated sequence types found in our study once more reveals the necessity of studying the role of transmission from animals to humans or vice versa.

SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S0950268816001412.

DECLARATION OF INTEREST

None.

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