calculated by competition experiments or after pre-incubation (20 min) with the inactivator, using the same reporter substrate. Inactivation by clavulanic acid was observed only after pre-incubation competitive assays (indirect ICSₐ 50 = 56 μM). On the other hand, 3-phenyl boronic acid showed enzymatic inactivation in both competitive and pre-incubation assays at high concentrations (direct ICSₐ 50 = 390 μM and indirect ICSₐ 50 = 151 μM).

INO-1 seems to be a cephalosporinase compatible with β-lactamases belonging to group 1 of the functional classification scheme. Although INO-1 may not explain by itself all the observed resistance to β-lactams in the clinical isolate of *I. limosus*, it contributes to the overall increase in MICs for the INQ-1-producing *E. coli* clone, even if transcriptional and post-transcriptional impairments are due to the unusual start codon and high GC.

**Nucleotide sequence accession number**
The nucleotide sequences of the blaINO-1 gene and neighbour genes of *I. limosus* MP06 have been assigned the EMBL accession number HG326253.

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**Transparency declarations**
None to declare.

**References**


**Emergence of extended-spectrum β-lactamase (ESBL) CTX-M-8 in Germany**

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**Keywords**: *Escherichia coli*, *Salmonella*, community acquired, food, antimicrobial resistance

Sir,

Extended-spectrum β-lactamases (ESBLs) of the CTX-M family are the main cause of resistance to third-generation cephalosporins in *Enterobacteriaceae*. The localization on transferable plasmids facilitates the fast spread of ESBL genes. Here we report on molecular analyses of CTX-M-8-positive enterobacterial isolates from Germany emerging between 2009 and 2012. Eight phenotypically ESBL-positive isolates of species *Escherichia coli* (n = 4), *Salmonella enterica* (n = 3; serovars *Agora*, *Newport* and *Anatum*) and *Enterobacter aerogenes* (n = 1) were investigated. These isolates were identified as part of routine diagnostics or within different studies of the interdisciplinary national research project RESET, which compares ESBL-producing enterobacterial isolates from humans, animals, food and the environment. The seven isolates from human samples and one from a food sample were from different regions in Germany (Table 1).

Susceptibilities to 11 antibiotics were determined by microbroth dilution according to CLSI criteria. The presence of β-lactamase genes (*bla*TEM-type, *bla*SHV-type and *bla*CTX-M-type) was tested by PCR and sequencing. In addition, PCR screening for plasmid-mediated quinolone resistance (PMQR) genes was performed. The identity of
Characteristics of CTX-M-8-producing Enterobacteriaceae isolates from Germany, 2009–12

| Species          | Year | Federal State | Material   | Infection/colony | bla<sub>CTX-M-8</sub> plasmid | repI<sub>1</sub>, ardA<sub>2</sub>, trbA<sub>5</sub>, sogS<sub>10</sub>, pilL<sub>10</sub> | Further plasmids | E. coli type | PFGE type | E. coli phylogroup |  |
|------------------|------|---------------|------------|------------------|-----------------------------|-----------------------------------------------------------------|----------------|--------------|------------|------------------|  |
| E. coli          | 2011 | BB            | urine      | infection (ambulant) | bla<sub>CTX-M-8</sub>, TEM-1 | 80 kb (IncI1, ST113) | — | — | — | — | — |
| E. coli          | 2011 | BB            | rectal swab | colonization | CTX-M-8, TEM-1 | 80 kb (IncI1, ST113) | — | — | — | — | — |
| E. coli          | 2012 | BB            | urine      | infection (ambulant) | CTX-M-8, TEM-1 | 80 kb (IncI1, ST113) | — | — | — | — | — |
| Salmonella       | 2011 | BB            | stool      | infection (ambulant) | CTX-M-8, TEM-1 | 80 kb (IncI1, ST113) | — | — | — | — | — |
| Salmonella       | 2009 | Thuringia     | stool      | infection (ambulant) | CTX-M-8, TEM-1 | 80 kb (IncI1, ST113) | — | — | — | — | — |
| Salmonella       | 2012 | LS            | stool      | infection (ambulant) | CTX-M-8, TEM-1 | 80 kb (IncI1, ST113) | — | — | — | — | — |
| Salmonella       | 2011 | Mecklenburg-Western Pomerania | stool | infection (ambulant) | CTX-M-8, TEM-1 | 80 kb (IncI1, ST113) | — | — | — | — | — |
| Salmonella       | 2011 | Lower Saxony  | stool      | infection (ambulant) | CTX-M-8, TEM-1 | 80 kb (IncI1, ST113) | — | — | — | — | — |
| Salmonella       | 2011 | Hamburg      | stool      | infection (ambulant) | CTX-M-8, TEM-1 | 80 kb (IncI1, ST113) | — | — | — | — | — |
| Salmonella       | 2011 | Mecklenburg-Western Pomerania | stool | infection (ambulant) | CTX-M-8, TEM-1 | 80 kb (IncI1, ST113) | — | — | — | — | — |
| Salmonella       | 2011 | Mecklenburg-Western Pomerania | stool | infection (ambulant) | CTX-M-8, TEM-1 | 80 kb (IncI1, ST113) | — | — | — | — | — |
| Salmonella       | 2011 | Mecklenburg-Western Pomerania | stool | infection (ambulant) | CTX-M-8, TEM-1 | 80 kb (IncI1, ST113) | — | — | — | — | — |

BB, Berlin-Brandenburg; H, Hesse; LS, Lower Saxony; Thuringia; MP, Mecklenburg-Western Pomerania.

ApMLST—IncI1 loci ST113: repI<sub>1</sub> (1), ardA<sub>2</sub> (2), trbA<sub>5</sub> (5), sogS<sub>10</sub>, pilL<sub>10</sub> (10); IncI1 loci ST114: repI<sub>1</sub> (1), ardA<sub>2</sub> (2), trbA<sub>5</sub> (8), pilL<sub>10</sub> (1), repI<sub>1</sub> (4), ardA<sub>2</sub> (2), trbA<sub>5</sub> (5), pilL<sub>10</sub> (10).

All eight isolates were cephalosporin resistant, but imipenem/meropenem susceptible. Three E. coli isolates were resistant to sulfoxmethoxazole/trimethoprim and ciprofloxacin. The E. aerogenes isolate was resistant to cefoxitin, and two Salmonella isolates and the E. aerogenes isolate showed increased MICs of ciprofloxacin (1 mg/L). PMQR genes were not found, indicating the presence of other mechanisms of quinolone resistance.

Molecular analyses revealed the presence of bla<sub>TEM-type</sub> genes in the E. coli isolates (Table 1), and in all isolates the ESBL gene bla<sub>CTX-M-8</sub> was identified including insertion sequence IS10 located upstream of the gene, as previously described. Three of the four E. coli isolates belonged to phylogenetic group B1. However, the XbaI macrorestriction analysis (PFGE) revealed different PFGE types confirming no genetic relatedness of the E. coli isolates (Table 1).

By brothmate conjugation, the bla<sub>CTX-M-8</sub> genes were transferred into an E. coli K12 J53 recipient. Transferred plasmids carrying bla<sub>CTX-M-8</sub> were ~80–85 kb in size and positive for replicon type IncI1. Since transconjugants were resistant only to ampicillin and cephalosporins, other resistance determinants did not seem to be located on the bla<sub>CTX-M-8</sub>-carrying plasmids. Plasmid analyses (S1-nuclease PFGE) demonstrated the presence of further plasmids in three E. coli isolates, and in one transconjugant a bla<sub>TEM-type</sub>-carrying plasmid (140 kb, IncI1/FIB) was co-transferred (Table 1 and Figure S1 (available as Supplementary data at JAC Online)).

Further subtyping of the bla<sub>CTX-M-8</sub> carrying IncI1 plasmids using plasmid multilocus sequence typing (pMLST) revealed three IncI1 subtypes. Six bla<sub>CTX-M-8</sub>-carrying plasmids had an identical IncI1 subtype with loci repI<sub>1</sub> (1), ardA<sub>2</sub> (2), trbA<sub>5</sub> (5), sogS<sub>10</sub>, pilL<sub>10</sub> (10) and pilL (10) that was assigned to the new IncI1 pMLST type ST113 (http://pubmlst.org/plasmid/). The bla<sub>CTX-M-8</sub>-carrying plasmid ST115 of the Salmonella Agona isolate differed in only one allele from ST113 (ardA<sub>4</sub> instead of ardA<sub>2</sub>, due to one nucleotide difference). The bla<sub>CTX-M-8</sub>-positive plasmid ST114 of isolate E. coli 3 shared three identical loci with ST113, but pMLST database results showed similarity (four identical loci, with a difference only in the sogS gene due to one nucleotide difference) to an 80 kb IncI1 bla<sub>CTX-M-8</sub>-carrying plasmid (ST60) from S. enterica serovar Virchow isolated in Germany 2007. Restriction fragment length polymorphism (RFLP) analyses revealed similar RFLP patterns for all the bla<sub>CTX-M-8</sub>-carrying plasmids (Figure S2, available as Supplementary data at JAC Online). We identified only minor differences (1–4 bands) between the plasmids of ST113, ST114 and ST115, indicating a close genetic relatedness.

Since its first description in 2000 in Brazil, CTX-M-8 has been identified in various enterobacterial species. A finding of CTX-M-8 in Salmonella as part of the present study has not been described before. Furthermore, data on bla<sub>CTX-M-8</sub>-carrying plasmids are rare, e.g. a 75 kb plasmid reported from Brazil, and IncI1/IncL/M plasmids were identified in Uruguay. Remarkably, CTX-M-8-producing strains were previously isolated from various sources including patients from hospitals, outpatients and food. We found CTX-M-8-positive isolates as colonizers of the human gut, as a cause of salmonellosis or urinary tract infections...
and in minced meat. Considering the similar bla\text{CTX-M-8}\textsuperscript{-}carrying plasmids in our isolates, we suppose that the transmission of CTX-M-8-producing Enterobacteriaceae might be possible via contaminated food and subsequent spread within the community facilitated by conjugal plasmid transfer. On the other hand, a possible transmission and introduction of CTX-M-8-positive strains to Europe by travellers or contaminated food can be assumed based on recent findings from Spain and the USA.\textsuperscript{12,13} However, due to a lack of epidemiological data, we cannot prove this hypothesis in the present study.

In conclusion, the rare ESBL type CTX-M-8 occurs occasionally in Germany, mainly in \textit{E. coli}, but also in \textit{Salmonella} and \textit{E. aerogenes}. The location of \textit{bla\text{CTX-M-8}} on very similar conjugative plasmids (~80–85 kb, IncI1) enhances the dissemination in Enterobacteriaceae.

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Transparency declarations

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Supplementary data

Figures S1 and S2 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References


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\textbf{In vitro activity of temocillin against multidrug-resistant clinical isolates of \textit{Escherichia coli}, \textit{Klebsiella} spp. and \textit{Enterobacter} spp., and evaluation of high-level temocillin resistance as a diagnostic marker for OXA-48 carbapenemase}

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\textbf{Keywords:} Enterobacteriaceae, ESBLs, KPC, NDM, VIM

Sir,

Temocillin is a narrow-spectrum penicillin active primarily against Enterobacteriaceae and resistant to hydrolysis by penicillinases, extended-spectrum \beta-lactamases (ESBLs), and AmpC enzymes.\textsuperscript{5} It has been shown also to retain some activity in vitro against