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Emergence and genetic environment of CTX-M enzymes produced by clinical *Escherichia coli* isolates in Germany, 2005-2009

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Introduction and Purpose

CTX-M enzymes are the predominant extended spectrum β-lactamases (ESBL) in *Escherichia coli* and other Enterobacteriaceae worldwide. As yet, more than 120 different CTX-M enzymes have been described [1]. bla_{CTX-M} genes are mostly located on plasmids and mobile genetic elements like IS*Ecp1* are responsible for the mobilization of several bla_{CTX-M} genes [2]. Both elements are involved in the dissemination and expression of CTX-M enzymes.

The objective of this study was to evaluate the prevalence of CTX-M type ESBLs among German *E. coli* isolates between 2005 and 2009. Additionally, CTX-M producing isolates collected in 2005 were analysed regarding the transferability of the bla_{CTX-M} gene and the presence of IS*Ecp1*.

Methods

Bacterial strains

300, 292 and 297 isolates consecutively collected in 15 German laboratories during three multicentre studies conducted in 2005, 2007 and 2009 respectively, were included into the study. Only first isolates were considered.

Susceptibility testing

Susceptibility to antimicrobial agents was determined by the broth microdilution procedure according to the DIN ISO standard [3]. EUCAST clinical breakpoints or epidemiological cut-off values were applied for interpretation, if available [4]. Resistance (R) to sulfamethoxazole was defined according to the breakpoint established by the Clinical and Laboratory Standards Institute (CLSI) [5].

ESBL screening test and isoelectric focusing (IEF)

ESBL-producing organisms were identified according to CLSI criteria [4] and further characterized by (IEF) as described by Bauernfeind et al. [6].

PCR assays and sequencing

DNA was isolated by using the Universal DNeasy® Tissue Kit and the Qiagen Plasmid Midi Kit (both: Qiagen, Hilden, Germany). bla_{CTX-M} genes and promotor regions of bla_{CTX-M} genes were analysed by PCR and sequencing.

Transferability of plasmids

Conjugation experiments with CTX-M producing isolates were done with *E. coli* J53 as recipient strain. Transconjugants were selected using cefotaxime (4 mg/l) and sodium azide (200 mg/l). Plasmids of non-conjugative strains were transformed into competent *E. coli* DH5-alpha cells by electroporation.

Results

The percentage of CTX-M producing *E. coli* was 4.7% (14/300) in 2005, 11.6% (34/292) in 2007 and 11.4% (34/297) in 2009 **(Figure 1)**.

In 2005, 50% of the CTX-M producing *E. coli* harboured CTX-M-1, 35.7% CTX-M-15 and 14.3% CTX-M-14, while in 2007 26.5%, 55.9%, 8.8%, 2.9% and 2.9% of the CTX-M positive isolates produced CTX-M-1, -15, -14, -9 and -2, respectively. One of the 34 strains isolated in 2007 harboured two enzymes, CTX-M-15 plus CTX-M-14. In 2009, 44.1%, 50% and 5.9% of the isolates expressed CTX-M-1, -15 and -14, respectively **(Table 1)**.

bla_{CTX-M} genes of the 14 CTX-M producing strains collected in 2005 were located on plasmids **(Table 2)**. All but one $bla_{CTX-M-1}$ genes as well as one $bla_{CTX-M-14}$ gene were expressed by conjugative plasmids, while plasmids harbouring $bla_{CTX-M-15}$ genes were not transferable. IS*Ecp1* was found upstream the respective bla_{CTX-M} gene in 8 strains **(Table 2)**. A complete insertion sequence was detected in 7 strains, whereas in one strain it was interrupted by IS*26*. Cross R to aminogylcosides, sulfamethoxazole, trimethoprim, fluoroquinolones, tetracycline, chloramphenicol and cefoxitin was found in 13, 12, 12, 11, 10, 5 and 4 isolates, respectively, and most frequently observed in CTX-M-15 producing strains. R to cefotaxime was transferable in all strains harbouring conjugative plasmids, but co-transfer of R to other drug classes was confined to two strains **(Table 2)**.

Conclusions

- **Our data suggest that the rate of CTX-M producing strains among *E. coli* isolates doubled between 2005 and 2007, but remained unchanged between 2007 and 2009.**
- **CTX-M-1 was the primary CTX-M type in 2005, while CTX-M-15 predominated in 2007 and 2009.**
- **CTX-M-1 enzymes were mainly disseminated by conjugative plasmids, while the spread of CTX-M-15 enzymes seems to be often associated with IS*Ecp1*.**
- **Resistance to other drug classes was more frequently distributed among CTX-M-15 than CTX-M-1 and CTX-M-14 producing isolates as it was previously shown for German *E. coli* isolates by Cullik et al. [7].**

Table 1: Prevalence of different CTX-M enzymes

CTX-M-type	pI	Number of isolates		
		2005	2007	2009
CTX-M group I		**12**	**28**	**32**
CTX-M-1	8.8	7	9	15
CTX-M-15	8.9	5	19	17
CTX-M group II			**1**	
CTX-M-2	7.9		1	
CTX-M group IV		**2**	**4**	**2**
CTX-M-9	8		1	
CTX-M-14	8.1	2	3	2
CTX-M groups I + IV			**1**	
CTX-M-15 + CTX-M-14	8.9, 8.1		1	
Total		**14**	**34**	**34**

Figure 1: Frequency of clinical *E. coli* isolates expressing CTX-M ESBLs

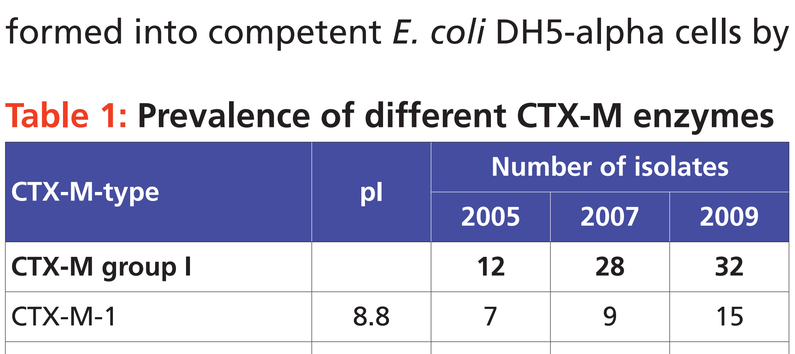

Table 2: Characteristics of CTX-M producing *E. coli* isoates collected in 2005

Strain No.	CTX-M-type	Located on plasmid	Plasmid transferability by		IS*Ecp1* upstream bla_{CTX-M}	Associated antibotic resistances*
			conjugation	transformation		
G30-30	CTX-M-15	+	-	-	+	CTX, GEN, KAN, TOB, CIP, NAL, SMX, TMP, TET, CHL
G23-39	CTX-M-15	+	-	-	+	CTX, FOX, AMK, KAN, STR, TOB, CIP, NAL, SMX, TMP, TET, CHL
G32-70	CTX-M-15	+	-	-	+**	CTX, FOX, GEN, KAN, STR, TOB, CIP, NAL, SMX, TMP, TET
G16-68	CTX-M-15	+	-	-	+	CTX, GEN, KAN, TOB, CIP, NAL, SMX, TMP, TET
G16-61	CTX-M-15	+	-	-	-	CTX, KAN, STR, TOB, CIP, NAL, SMX, TMP, TET
G11-43	CTX-M-1	+	-	-	+	CTX, STR, SMX, TMP, TET
G23-3	CTX-M-14	+	-	-	+	CTX, FOX, STR, CIP, NAL, SMX, TMP, TET
G11-54	CTX-M-14	+	+	n.t.	+	**CTX,** STR, NAL
G27-80	CTX-M-1	+	+	n.t.	-	**CTX, GEN, STR, TOB**
G32-74	CTX-M-1	+	+	n.t.	-	**CTX,** STR, NAL, SMX, TMP, TET, CHL
G32-72	CTX-M-1	+	+	n.t.	-	**CTX,** STR, CIP, NAL, SMX, TMP, TET
G18-53	CTX-M-1	+	+	n.t.	-	**CTX,** CIP, NAL, SMX, TMP, CHL
G18-52	CTX-M-1	+	+	n.t.	+	**CTX,** FOX, STR, CIP, NAL, **SMX, TMP,** CHL
G22-81	CTX-M-1	+	+	n.t.	-	**CTX,** STR, SMX, TMP, TET

* Antibiotic resistances detected in transconjugants are given in bold; ** interrupted by IS26; n.t., not tested
CTX = cefotaxime; FOX = cefoxitin; AMK = amikacin; GEN = gentamicin; KAN = kanamycin; STR = streptomycin; TOB = tobramycin; CIP = ciprofloxacin; NAL = nalidixic acid; SMX = sulfamethoxazole; TMP = trimethoprim; TET = tetracycline; CHL = chloramphenicol

References

1. Lahey clinics. http://www.lahey.org/studies/webt.asp; Last visit of the website on March 12th, 2012
2. Eckert C et al. (2006) J. Antimicrob Chemother 57: 14-23
3. DIN (2006) ISO/FDIS 20776-1:2006
4. EUCAST (2012) http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/Breakpoint_table_v_2.0_120221.pdf
5. CLSI (2012) M100-S22
6. Bauernfeind A et al. (1999) Antimicrob Agents Chemother 43: 1924-31
7. Cullik A et al. (2010) J Med Microbiol 59: 580-7