PREVALENCE AND MOLECULAR EPIDEMIOLOGY OF CTX-M β-LACTAMASES FROM CROATIA

B. Bedenić¹, M. Tonkić², I. Jajić-Benčić³, S. Kalenić⁴, A. Baraniak⁵, J. Fiett⁵, M. Gniadkowski⁵ ¹Department of Microbiology, School of Medicine, University of Zagreb, Zagreb, Zagreb, Croatia, ²Department of Microbiology, University Hospital Split, ³Department of Microbiology, Sisters of Mercy Hospital, Zagreb, ⁴Department of Clinical and Molecular Microbiology, Clinical Hospital Center Zagreb, ⁵ National Institute of Public Health, Warshaw, Poland

BACKROUND AND AIM

-CTX-M extended-spectrum β -lactamases (ESBLs) have been the source of the most spectacular and striking changes in ESBL epidemiology in recent years.

-Identified first at the end of the 1980s, for almost the whole next decade they were considered in general as rather rare ESBL types.

-Recently *E. coli* strains with unusual resistance phenotype conferring very high resistance to cefotaxime and ceftriaxone have been isolated in three University hospitals in Croatia and Zagreb Institute of Public Health.

-The aim of this study was to characterize these isolates which turned out to be the first CTX-M producers identified in Croatia

MATERIALS AND METHODS

Bacteria

Fourteen strains with unusual resistance phenotype were collected from Clinical Hospital Center Zagreb, Sisters of Mercy Hospital Zagreb, University Hospital Split, and Zagreb Institute of Public Health. Detection of *B*-lactamases

Extended-spectrum β -lactamases (ESBL) were detected by double-disk synergy test and CLSI combined disk test.

Antibiotic susceptibility testing

The susceptibility to a wide range of antibiotics was determined by broth microdilution method according to CLSI. The transferability of the resistance determinants was determined by broth mating method.

Characterization of β-lactamases

ESBLs were characterized by isoelectric focusing, substrate profile determination, polymerase chain reaction and sequencing of *bla*CTX-M genes.

Molecular typing of the E. coli isolates

Genetic relatedness between the strains was tested by pulsed-field gel electrophoresis.

RESULTS

Antibiotic susceptibilities

The study isolates were the first *E. coli* ESBL-producing isolates identified as highly resistant to cefotaxime (MICs, 64 - >1024 mg/L) and ceftriaxone (MICs, 128 - >1024 mg/L) in the three Croatian hospitals.

The prevalence of this resistance phenotype among *E. coli* isolates in the University Hospital in Split was 5% (5/100), 2.5% in the Clinical Hospital Center in Zagreb (5/200), 2.5% (1/50) in the Sisters of Mercy Hospital in Zagreb and 2.8% (3/105) in the community patients from Zagreb. Despite some similarities, the susceptibility testing revealed significant differences between the groups of isolates identified in the three hospitals (Table 1). The isolates from the University Hospital Center in Zagreb were almost uniform in their resistance patterns and levels, and they were in general more resistant to the antimicrobials tested than the remaining isolates.

RESULTS



Characterization of β-lactamases

All of the isolates produced β -lactamases with high pl values, either 8.9 in the case of the isolates from the University Hospital Center in Zagreb or 8.4 in the case of the all remaining isolates (Table 2, Figure 1). All of the *E. coli* isolates yielded amplicons of the expected sizes of around 500 bp with primers MA-1 and MA-2 (Figure 2), and of around 1 kb with primers P1C and P2D. Sequencing of *bla*CTX-M genes revealed the presence of the CTX-M-3 β-lactamase in all clinical isolates from Split and the Sisters of Mercy Hospital in Zagreb, whereas CTX-M-15 was found in the isolates from the Clinical Hospital Center in Zagreb. The two community isolates produced CTX-M-14 β-lactamase (Figure 3). The mobile genetic element IS26 was found in all of the

isolates from Split.

Strain No	Specimen	Institution ¹	PFGE type	Frequency of conjugation	Cotransferred resistance markers ²	Isoelectric point	Type of enzyme
16	urine	1	1	10.4	An, Gm	8.4	CTX-M-3
32	bronchoaspirate	1	2	10.4	т	8.4	CTX-M-3
36	urine	1	3	10.4	т	8.4	CTX-M-3
86	urine	1	2	10-7	т	8.4	CTX-M-3
100	urine	1	4	10.4	An, Gm, Smx	8.4	CTX-M-3
49	urine	2	6	1	1	8.4	CTX-M-3
52	Blood culture	3	5	10.2	An, Gm, T, Smx	8.9	CTX-MI
1059	Blood culture	3	5	10.7	An, Gm, T, Sms	8.9	CTX-MI
1830	Blood culture	3	5	10.7	An, Gm, T, Sms	8.9	CTX-MIS
4274	Blood culture	3	5	10.7	An, Gm, T, Smx	8.9	CTX-MI
4874	Blood culture	3	5	10.7	An, Gm, T, Sms	8.9	CTX-MI
19	Urine	4	7	1	1	8.4	
22	Urine	4	8	5x10-*	1	8.4	CTX-M-1
34	Urine	4	2	1	1	8.4	CTX-M-1



RESULTS

Molecular characterization of the isolates

The all five CTX-M-15-producing isolates from the University Hospital Center in Zagreb were indistinguishable in the PFGE analysis, and different from the all remaining isolates (Figure 3). The group of CTX-M-3 producers from the University Hospital in Split was much more diverse with only two isolates (isolates 32 and 36) that were clonally related to each other, and the unique remaining ones. The isolate from the Sisters of Mercy Hospital in Zagreb was unrelated to any other study isolate. Furthermore, the community isolates were distinct between themselves and from the hospital isolates.

Figure 3. Pulsed field electrophoresis of genomic DNA



CONCLUSIONS

 So far all of the studies on ESBL-producing organisms in Croatia have shown the high prevalence of enzymes of the SHV family, such as SHV-5 and SHV-12.

•From the beginning of the 2000s, CTX-Ms seem to have become the major type of ESBLs all over Europe, and Croatia belongs to the countries in which their presence has been observed at the latest. •Although they are rather still rare, the identification of CTX-M producers in three hospitals in two distant cities suggests that the problem has already started to exist.

•It is not striking that the up o enzyme variants identified in Croatia were CTX-M-3 and CTX-M-15. Both the β -lactamase belong to the CTX-Ms spread world-wide and seem to be predominant in Europe •Our data point out that CTX-M producing *E. coli* are widely scattered in Croatia, and that the underlying epidemiology involves not only the spread of strains between the centers but also instances of intra-hospital spread and horizontal transfer of plasmids carrying *bla*CTX-M genes. •The preliminary data on the genetic context of the Croatian *bla*CTX-M genes suggest that the *bla*CTX-M-15 genes in the isolates from the University Hospital Center in Zagreb are like those common for the wider part of Europe and not for Poland. However, the *bla*CTX-M-3 genes from Split and the other hospital in Zagreb seem to be unrelated to them.

 Despite the high similarity of these enzymes, differences in the genetic context of their genes indicated that they emerged independently on each other. Moreover, it is possible that there were also two different origins of CTX-M-3, which altogether is striking considering the relative small geographic region of the isolates identification

•Urinary tract was the most important source of CTX-M producing *E. coli* in our study particulary for community isolates which is in concordance with the reports for other authors.

•The detection of CTX-M β -lactamase s in Croatia raises the question whether such enzymes arise de novo in multiple geographic regions, or alternatively, are transmitted across national borders. The importance of these questions emphasizes the need for global surveillance of antibiotic resistance so that appropriate control strategies can be designed and implemented