Antimicrobials: resistance surveillance
Carbapenemases in urinary isolates of Enterobacteriaceae from Croatia

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BACKGROUND
Recently, emergence of carbapenem-resistance was observed among urinary isolates in University Hospital Center Zagreb. The aim of the study was to characterize the carbapenem-resistance mechanisms of the tested strain in order to analyze the molecular epidemiology of carbapenem resistance in Enterobacteriaceae from Croatia.

MATERIAL AND METHODS
The antimicrobial susceptibility to a wide range of antibiotics was determined by broth microdilution method. Double-disk-synergy test (DDST) was performed to detect ESBLs and modified Hodge test (MHT) was used to screen for production of carbapenemases. MBL E-test was used to screen for production of metallo-β-lactamas (MBLs). Additionally the isolates were tested by combined disk tests using four disks of meropenem or imipenem one without and the other three with 3-aminocephaloronic acid (PBA) test with 0.1 M EDTA or both PBA and EDTA to screen for KPC, MBLs or simultaneous production of MBL and KPC respectively. The presence of genes encoding broad and extended-spectrum β-lactamases (\textit{bla}\textsubscript{SHV}, \textit{bla}\textsubscript{TEM}, \textit{bla}\textsubscript{CTX-M} and \textit{bla}\textsubscript{PER-1}), plasmid-mediated AmpC β-lactamases, group A carbapenemases (\textit{bla}\textsubscript{KPC}, \textit{bla}\textsubscript{SME}, \textit{bla}\textsubscript{IMI}, \textit{bla}\textsubscript{NMC}), metallo β-lactamases (\textit{bla}\textsubscript{VIM}, \textit{bla}\textsubscript{IMP} and \textit{bla}\textsubscript{NDM}), and carbapenem hydrolyzing oxacillinases (\textit{bla}\textsubscript{OXA-48}) was determined by PCR.

RESULTS
Five centers participated in the study (University Hospital Center Zagreb, University Hospital Center Split, General Hospital Pula, University Hospital Center Sestre Milosrdnice and University Hospital Center Osijek). In total 49 strains (31 	extit{Enterobacter cloacae}, 1 one 	extit{Enterobacter amnigenus}, 11 	extit{Klebsiella pneumoniae}, 4 	extit{Citrobacter freundii}, and one 	extit{Escherichia coli} and 	extit{Serratia marcescens}, respectively) were isolated from urine during 2012-2013 from two large hospital centers in Croatia. All strains were resistant to amoxyccilin alone and combined with clavulanate, cefuroxime, ceftazidime, cefotaxime, ceftriaxone but susceptible to colistin. The strains showed variable susceptibility/resistance patterns to cefepime, imipenem, meropenem and ertapenem.

VIM-1 was found in 36 strains, NDM-1 in one and KPC-2 in two, while one strain coharboured VIM-1 and NDM-1. Nine strains were negative for true carbapenemases and harboured only ESBL belonging to CTX-M family. VIM-1 β-lactamase was carried by 	extit{E. cloacae}, 	extit{K. pneumoniae}, 	extit{C. freundii} and 	extit{S. marcescens}. NDM-1 was carried by only 	extit{E. cloacae} whereas 	extit{K. pneumoniae} was the only species positive for KPC-2. The majority of MBL positive strains coharboured TEM-1 and CTX-M-15 beta-lactamase while both KPC-2 coproduced TEM-116 beta-lactamase. Plasmids encoding VIM- MBLs belonged to widespread IncA/C or IncL/M PBRT.

CONCLUSIONS
The study demonstrated emergence of carbapenemase-producing Enterobacteriaceae associated with urinary tract infections. The predominance of VIM-1 and KPC-2 β-lactamase was observed. In the previous studies 	extit{Enterobacteriaeae} positive for ESBLs belonging to CTX-M family were found to be dominant multiresistant pathogen among urinary isolates but shift to carbapenemase producers was noticed in the last two years. The study found significant emergency of carbapenemase-producing organisms causing urinary tract infections which could pose a serious therapeutic challenge.