Objectives
The first carbapenem-resistant strain of *Klebsiella pneumoniae* was isolated in 2008 in University Hospital Center in Zagreb. A remarkable increase in the number of carbapenem-resistant isolates was observed in 2011 and 2012. The carbapenem resistance of these isolates was analysed and VIM-1 was found to be the most prevalent type, followed by NDM-1 and KPC-2. In 2013 an increasing trend of carbapenem-resistant Enterobacteriaceae was observed. The aim of the study was to analyse the carbapenem-resistance and the molecular epidemiology of these strains.

Methods
In total 49 carbapenem non-susceptible strains of Enterobacteriaceae were collected from January till October 2013 from three hospital centers located in different geographic regions in Croatia. The strains were identified by Vitec automated system. The antimicrobial susceptibility to a wide range of antibiotics was determined by broth microdilution method according to CLSI. Double-disk-synergy test (DDST) was performed to detect ESBLs and modified Hodge test (MHT) was used to screen for production of carbapenemases. The presence of genes encoding broad and extended-spectrum β-lactamases (*bla*<sub>SHV</sub>, *bla*<sub>TEM</sub>, *bla*<sub>CTX-M</sub> and *bla*<sub>PER-1</sub>), plasmid-mediated AmpC β-lactamases, group A carbapenemases (*bla*<sub>KPC</sub>, *bla*<sub>SME</sub>, *bla*<sub>IMI</sub>, *bla*<sub>NDM</sub>), metallo-β-lactamases (*bla*<sub>VIM</sub>, *bla*<sub>IMP</sub> and *bla*<sub>NDM</sub>), and carbapenem hydrolyzing oxacillinases (*bla*<sub>OXA-48</sub>) was determined by PCR.

Results
The majority of the isolates originated from haematology and bone marrow transplantation wards. All strains were resistant to amoxycillin alone and combined with clavulanate and piperacillin with tazobactam, cefuroxime, cefazoline, cefotaxime, ceftriaxone and ertapenem. Above 90% of the strains were resistant to imipenem, meropenem and cefoxitin. Only one strain was resistant to colistin. Amikacin and ciprofloxacin demonstrated moderate activity with 39% and 33% of resistant strains, respectively. All strains were positive in phenotypic tests for ESBLs while modified Hodge test was positive in all but four strains. Twenty-five strains were positive for *bla*<sub>VIM-1</sub>, 16 for *bla*<sub>KPC-2</sub> and two for *bla*<sub>NDM-1</sub>. Twenty-three strains coharboured *bla*<sub>TEM</sub> and 14 *bla*<sub>CTX-M</sub> genes. Two strains were found to coproduce VIM-1 and NDM-1 (C. freundii and E. cloacae). Four strains were positive only for CTX-M β-lactamases. The production of KPC β-lactamases was associated with high level of resistance to all three carbapenems in contrast to VIM and NDM producing organisms which showed variable level of resistance to carbapenems.

Conclusions
The study found the persistence of VIM-1 and NDM-1 among our Enterobacteriaceae. To our knowledge this is the first report of coproduction of VIM-1 and NDM-1 and of the emergence of colistin resistance among Enterobacteriaceae from Croatia. Loss of colistin activity against carbapenem-resistant Enterobacteriaceae can pose a serious therapeutic problem because there are no options left for the treatment of infections associated with such isolates. Carbapenemase encoding genes are located on mobile genetic elements and thus their accurate detection is of uppermost importance.