DIVERSITY OF CARBAPENEMASES IN CLINICAL ISOLATES OF ENTEROBACTERIACEAE IN CROATIA: THE RESULTS FROM THE MULTICENTER STUDY

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Introduction and purpose

The first carbapenem-resistant strain of Klebsiella pneumoniae in Croatia was isolated in 2008 at University Hospital Center in Zagreb, producing metallo-β-lactamase (MBL) NDM-1. Since that Enterobacteriaceae with reduced susceptibility to one or more carbapenem emerged sporadically in different geographic regions in Croatia. The first group A carbapenem found in Croatia was KPC-2 identified in K. pneumoniae strain from University Hospital Center in Zagreb at the beginning of 2011. A remarkable increase in the number of carbapenem-resistant isolates was observed in 2011. This observation gave rise to a multicenter study on carbapenem-resistant Enterobacteriaceae from Croatia. The aims of this study were the characterization of the mechanisms of resistance at the molecular level, genotyping of the strains, and the epidemiological analysis of carbapenem-resistant Enterobacteriaceae from Croatia.

Material and methods

In total 57 carbapenem non-susceptible strains of Enterobacteriaceae (33 Enterobacter cloacae, 13 K. pneumoniae, one O. pyogenes, eight Citrobacter freundii, one Serratia marcescens and one Escherichia coli) were collected during 2011-2012 from five hospital centers located in different geographic regions in Croatia (University Hospital Center Zagreb, Sisters of Mercy Hospital Zagreb, University Hospital Otok, University Hospital Split and County Hospital Pula). The strains were identified by conventional biochemical testing and by Vitek automated system (bioMérieux, Marcy l’Etoile, France). MBL-β-lactamase was detected by modified Hodge test and extended-spectrum β-lactamase by using combined disk test with phenylboronic acid and carbapenems was positive in one strain as typical for KPC while DDST and combined disk test with clavulanic acid were positive in all but three strains indicating the production with resistance rate of 14%. Colistin was the most potent antibiotic with MIC ≤ 1 μg/mL (7/57 strains). Ampicillin-resistant producers of metallo-β-lactamase were selected and isolated from the 52/57 strains. The enzyme production was confirmed by combined disk test with clavulanic acid and positive results were detected with clavulanic acid and clavulanic acid combined with ticarcillin. The isolates were sequentially cultured, and several extra isolates were isolated and confirmed by DDST and combined disk test with clavulanic acid

Conclusions

The first carbapenem-resistant K. pneumoniae was reported from Croatia in 2008. The true carbapenemases emerged in significant number from 2011. Carbapenemases found in this study belonged to KPC and VIM family. K. pneumoniae strains with KPC carbapenemases were first detected in North Carolina in 2003. Then, the East of China was identified as a potential region for the spread of KPC-producing bacteria. Since then, the number of KPC-producing bacteria have spread widely in Israel, Greece and Italy. The first report of KPC-β-lactamases in Croatia originated from 2011 from University Hospital Center Zagreb; it was a single K. pneumoniae isolate and effective infection control measures means the strain did not spread throughout the hospital. The second case was a K. pneumoniae strain from Sisters of Mercy Hospital in 2012. The patient was previously treated in Germany. Therefore, upon admission to hospital, screening for multiresistant organisms should be performed in patients that have received medical care in other countries, particularly in endemic regions. This strain, like the previous one from 2011 did not carry other resistance genes except of blaoxa-2, which was previously found in E. coli from Zagreb, Croatia. The carbapenemases were detected in isolates of the same strain with the same plasmid and MIC values. The coexistence of carbapenemases in the different hospitals in Croatia gives evidence to the multicenter study from Croatia.