Emergence of OXA-48 beta-lactamase in Enterobacteriaceae in Croatia

Dijana Varda-Brkic1, Natasa Beader2, Marko Cacic3, Mirna Vranic-Ladavac4, Sandra Sardelic5, Ines Jajic6, Andrea Grisold7, Gernot Zarfel8, Josefa Luxner9, Zrinka Bosnjak10, Branka Bedenic10

1University Hospital Zagreb, University Hospital Zagreb, Clinical Department for Clinical and Molecular Microbiology, Zagreb, Croatia

2School of Medicine, University of Zagreb, University Hospital Center Zagre, Clinical Department for Clinical and Molecular Microbiology, Zagreb, Croatia

3St Antonius Hospital, Cardiology, Kleve, Germany

4Public Health Institute of Istria County, Microbiology, Pula, Croatia

5University Hospital Center Split, University Hospital Center Zagreb, Microbiology, Split, Croatia

6University Hospital Center Sisters of Fmercy, University Hospital Center Sisters of Mercy, Microbiology, Zagreb, Croatia

7Institute for Microbiology, Hygene and Environenmental Medicine, University of Graz, Graz, Austria

8Medical University of Graz, Graz, Austria

9University of Graz, Graz, Austria

10School of Medicine, University of Zagreb, University Hospital Center Zagreb, Clinical Department for Clinical and Molecular Microbiology, Zagreb, Croatia

Background: Previous studies found VIM-1, NDM-1 and KPC-2 among CRE in Croatia with VIM-1 being the most prevalent. Recently OXA-48 was identified in three hospital centers.

Material/methods: Since 2012 surveillance system for CRE was implemented in University Hospital Center (UHZ) in Croatia which included phenotypic and molecular identification of carbapenemases in Enterobacteriaceae. In total four carbapenem non-susceptible strains of K. pneumoniae were found to be positive for OXA-48 in three hospital centers (University Hospital Center Zagreb-UHZ, University Hospital Center Sisters of Mercy-UHZ, University Hospital Center Split-UHS). The antimicrobial susceptibility 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 metallo-β-lactamases (MBLs). Additionally the isolates were tested by combined disks test with EDTA or 3-aminophenylboronic to screen for KPC, MBLs or simultaneous production of MBL and KPC respectively. The transferability of meropenem resistance was determined by conjugation employing E. coli A15R strain resistant to sodium-azide. The presence of genes encoding broad and extended-spectrum β-lactamases (blaSHV, blaTEM, blaCTX-M and blaPER-1), plasmid-mediated AmpC β-lactamases, group A carbapenemases (blaKPC, blaSME, blaMBL).
metallo β-lactamases (blaVIM, blaIMP and blaNDM), and carbapenem hydrolyzing oxacillinases (blaOXA-48), was determined by PCR.

**Results:** The strains were uniformly resistant to amoxicillin alone and combined with clavulanate, cefazoline, cefuroxime, ceftazidime, cefotaxime, ceftriaxone, but uniformly susceptible to colistin and had variable resistance patterns to carbapenems. Modified Hodge test was positive indicating the production of carbapenemase. Phenotypic testing was positive for ESBL but negative for, MBL, KPC and AmpC. Meropenem resistance was not transferred to *E. coli* recipient strain. PCR revealed the strains to be positive also for CTX-M-15 and TEM-1. The strains from UHZ coproduced OXA-48 and VIM-1. Two strains from UHZ had identical rep-PCR patterns but different from those from UHS and UHM. The patients did not have a travel history to the countries where OXA-48 is endemic.

**Conclusions:** The study demonstrated emergence of OXA-48 β-lactamase in three hospital centers located in different geographic regions in Croatia. The strains from different hospitals displayed different rep-PCR patterns and thus it could be concluded that they occurred as independent events. OXA-48 was first reported in Turkey in 2001 but later spread in Mediterranean and western European countries such as Israel, France, Italy, Spain, Germany, Switzerland, Belgium, and the Netherlands. Most of the strains in western Europe were imported from Turkey, Morocco, Egypt, Algeria or Libya. Croatia was spared from this type of carbapenemases until 2014. In our study, there was no link to the endemic areas.