The purpose of this study was to report the identification of OXA-48 carbapenemase in seven ESBL positive E. coli clinical isolates, susceptible to carbapenems by disk-diffusion and E-test method, but with a borderline MIC value of ertapenem (1 mg/L). Unexpectedly, three of seven isolates were shown to harbour A/C plasmid, previously associated with VIM or NDM MBLs. This finding prompted us to analyse the mechanisms of reduced susceptibility to ertapenem.

Seven ertapenem non-susceptible E. coli originated from different anatomic sites were analyzed. The isolates were collected over 12 months period from patients hospitalized in GH Slavonski Brod, Slavonia region, Croatia. The susceptibility to a wide range of antibiotics was determined by disk-diffusion and broth microdilution method according to EUCAST. The transferability of cefotaxime resistance and reduced susceptibility to meropenem was determined by conjugation. Carbapenemases and extended-spectrum β-lactamases were phenotypically detected. The presence of bla_{CARB} (bla_{KPC}, bla_{VIM}, bla_{IMP}, bla_{NDM}, bla_{OXA-48}) and bla_{ESBL} genes (bla_{SHV}, bla_{TEM}, bla_{CTX-M}) was determined by PCR and sequencing. PCR-based replicon typing (PBRT) was applied to determine the plasmid content of the tested isolates. PFGE genotyping of XbaI-digested genomic DNA was performed with CHEF-DRIII system.

Isolates showed a high level of resistance to most of the antibiotics and were susceptible to colistin, amikacin, tigecycline, and fosfomycin. Despite susceptibility to ertapenem in disk-diffusion and E-test, dilution test yielded borderline MICs of 1 mg/L. Hodge and Carbapenem Inactivation Method test were positive indicating the production of carbapenemase.

All isolates transferred cefotaxime and meropenem resistance to E. coli recipient strain. The isolates were shown to possess group 1 of CTX-M β-lactamases and TEM–1 in addition to OXA-48. Bla_{OXA-48} genes were preceded by IS1999 whereas ISEcp was found upstream of bla_{CTX-M-15} genes in three isolates. PFGE analysis revealed two clusters and containing subgroups of highly related isolates.

This report points to the need for determination of carbapenem MICs in E. coli ESBL positive isolates and additional testing for all isolates with borderline ertapenem MIC defined by EUCAST. Unlike previous studies in Croatia, the dissemination of OXA-48 in Slavonia region was associated with highly related isolates, belonging into two clusters.