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OXA-244-producers, the nightmare for clinical microbiology labs: insights into their genomes

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Background: OXA-48-producing Enterobacteriaceae have widely disseminated throughout Europe. Different variants have been reported, differing by a few amino acid substitutions or deletions mostly in the region of the $\beta 5$ - $\beta 6$ loop. OXA-244 is a single point mutant derivative of OXA-48 displaying reduced carbapenemase activity. Here, we report features of OXA-244-producing *E. coli* that may help clinical laboratories to improve their capacity to detect this OXA-48 like carbapenemase. In addition, we have investigated the genetic background and the genetic relatedness of seven OXA-244-producing *E. coli* isolates from different geographical origins.

Material/methods: Seven OXA-244-producing *E. coli* isolates from our laboratory collection were analyzed. Susceptibility testing, whole genome sequencing, plasmid content, electroporation experiments, evaluation of selective culture media (ChromID[®] ESBL and ChromID[®] CARBA SMART, bioMérieux), biochemical detection of the carbapenemase activity (Carba NP test, RAPIDEC[®] CARBA NP (bioMérieux), a MALDI-TOF MS hydrolysis assay (Bruker)), immunochromatographic detection of the carbapenemase using OXA-48 K-SeT[®] (Coris bioconcept), molecular detection using Xpert[®] Carba-R v2 (Cepheid) and the in-house PCR, and molecular typing using rep-PCR (DiversiLab[®] system, bioMérieux) and MLST were performed.

Results: Four clones were identified among the seven OXA-244-producing *E. coli* isolates. All but one co-produced at least one extended spectrum β -lactamase and or an plasmid encoded cephalosporinase in addition to OXA-244, leading to their efficient detection on ChromID® ESBL medium (bioMérieux). At the opposite, only one isolate was able to grow on the ChromID® CARBA SMART medium (bioMérieux), a medium dedicated to the screening of carbapenemase-producing Enterobacteriaceae. The carbapenemase production was detected in 57.1 %, 71.4 %, 71.4 %, and 100% of the case using the Carba NP test, the RAPIDEC® CARBA NP (bioMérieux), a MALDI-TOF MS hydrolysis assay (Bruker), and OXA-48 K-SeT®(Coris bioconcept), respectively. Both molecular confirmatory tests, e.i. the Xpert® Carba-R v2 and the in-house PCR were able to detect *bla*_{OXA-244} gene in all *E. coli* isolates. The rep-PCR results showed that the seven isolates corresponded to four different clones corresponding to a different ST: ST-38, ST-361, ST-1722 and ST-3541. For all the clones an IS1R-made composite transposon harbouring the *bla*_{OXA-244} gene was integrated into the chromosome at the same position, thus supporting an insertion hotspot.

Conclusions: Our result indicate that OXA-244-producing *E. coli* isolates are difficult to detect, which in terms may lead to a silent spread of this type of enzyme, Despite reduced susceptibility to carbapenems and temocilin only one strain (14%) was able to grow in the ChromID® CARBA SMART medium. Screening procedures should be adapted in areas with high prevalence of OXA-244-producers.