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Abstract (publication only)

Phenotypic and molecular characterisation of CMY-46 and CMY-50, two novel plasmid-mediated AmpC beta-lactamase carried by Escherichia coli

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Objectives: The identification of isolates containing AmpC beta-lactamases is epidemiologically and clinically relevant. With this study we performed the phenotypic and molecular characterization of two new CMY-2-types, designated CMY-46 and CMY-50, encountered among a total of 1664 clinical non-duplicate isolates of various Enterobacteriaceae species. Methods: E. coli INSRA1169 and INSRA3413 were isolated from the urine of patients with 77 years and 7 months old, hospitalized in the ward and in pediatrics, respectively. The blaCMY genes were cloned in the plasmid pBK-CMV and transformed into electrocompetent E. coli DH5 alpha delta ampC by electroporation. Antimicrobial susceptibility (MIC) was determined by a microdilution method. E. coli INSRA6015, a CMY-2-producer, was used for phenotype comparison. PCR-mapping of the genetic environment of new blaCMY genes was performed using primers for known antibiotic and mercury resistance genes. Results: Antimicrobial susceptibly tests showed that all isolates and respective transformants were nonsusceptible to amoxicillin, amoxicillin plus clavulanic acid, cephalothin, cefoxitin, ceftazidime and cefotaxime. INSRA1169 and INSRA6015 were also nonsusceptible to ciprofloxacin and to trimethoprim. Regarding gentamycin, only INSRA1169 was resistant. Its noteworthy that the transformants EcDH5a(pBK-CMY-2) and EcDH5a(pBK-CMY-46) exhibited higher values for extended-spectrum cephalosporins than the respective isolates. All strains were susceptible to cefepime and imipenem, showing synergy between cloxacilin and cefoxitin and/or ceftazidime. No phenotypic alterations were found comparing the new CMY-type with the parental CMY-2. The genetic characterization of CMY-46 and CMY-50-encoding genes revealed a Citrobacter freundii chromosome-type structure, encompassing a blc-sugE-blaCMY-2-type-ampR platform in both isolates. In addition, a sul1-type class 1 integron and a truncated mercury resistance operon were encountered. Conclusion: Although the CMY-type enzymes studied conferred resistance to extended-spectrum cephalosporins, the susceptibility to cefepime lead us to assume that those enzymes are not extended-spectrum cephalosporinases. Otherwise, the presence of three genetic resistance-encoding regions is of great concern, namely the truncated mercury resistance operon, which may help to promote antibiotic resistance through indirect selection.