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Antimicrobials: Resistance surveillance

Coproduction of CTX-M-15 and CMY-2 in animal *Escherichia coli* isolate from Croatia

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Objectives: The aim of the study was to characterize the resistance to expanded-spectrum cephalosporins in animal isolate of Escherichia coli.
Methods: The antimicrobial susceptibility to a wide range of antimicrobials was determined by disk-diffusion and broth microdilution method according to CLSI guidelines and interpreted according to CLSI breakpoints . Double-disk synergy test (DDST) and combined disk test with addition of clavulanic acid and PBA (3-aminophenylboronic acid) was performed to detect ESBLs and plasmid-mediated ampC β-lactamases. Transferability of ceftazidime resistance was tested by conjugation. The presence of genes encoding broad and extended-spectrum β-lactamases, plasmid-mediated AmpC β-lactamases and fluoroquinolone resistance in clinical isolate and transconjugant strains was examined by PCR. Plasmid incompatibility group was determined by PCR-based replicon typing according to Carattoli et al.
Results: A female two year old non-castrated American Staffordshire terrier was admitted to a private veterinary clinic and a generalized demodicosis was suspected based on clinical findings. After laboratory confirmation of *Demodex canis* in skin scrapings, a combination of imidacloprid and moxidectin (Advocate, Bayer Animal Health) was prescribed. Over the course of two months and due to the suspected secondary bacterial skin infection, the dog was treated with oral amoxicillin/clavulanate (Klavocin, PLIVA, Croatia) and enrofloxacin (Enroxil, Krka, Slovenia) without success. Three months after the first admission, a clinical examination revealed a superficial pyoderma with numerous pustules on distal parts of all four extremities. *Escherichia coli* resistant to expanded-spectrum cephalosporins was isolated from a wound swab of a dog admitted at Veterinary clinic in Zagreb. The strain was resistant to third generation cephalosporins, cefoxitin and ciprofloxacin but susceptible to carbapenems and gentamicin. Resistance to expanded-spectrum cephalosporins was transferred to E. coli recipient strain by conjugation. PCR and sequencing revealed the production of plasmid-mediated AmpC β-lactamase CMY-2 and extended-spectrum β-lactamase CTX-M-15. IS*Ecp1* was not found upstream or downstream of the $bla_{\text{CTX-M}}$ gene. Plasmid extractions were positive for $bla_{\text{CTX-M}}$ and bla_{CMY} genes. Qnr genes were not found. The strain belonged to FIB repA PBRT.
Conclusions: To our knowledge this is the first report of coproduction of CTX-M-15 and CMY-2 β-lactamase in Croatia and first report of an ESBL and plasmid-mediated AmpC in animal specimen from Croatia. Previous studies found CTX-M-3 and CTX-M-15 to be the most prevalent types of ESBLs in human isolates of *E. col*i in Croatia. CMY variants were found as additional β-lactamases among carbapenemase producing Enterobacteriaceae in Croatia. Administration of expanded-spectrum cephalosporins in companion and food animals exerts selection pressure which favors horizontal spread of plasmids carrying bla_{ESBL} and bla_{ampC} genes and vertical spread of multiresistant strains.Screening of stool samples or rectal swabs should be performed in the future to analyze the faecal carriage of ESBL and plasmid-mediated AmpC producing *Enterobacteriaceae* in pet animals in Croatia.