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More and more third generation cephalosporin-resistent enteric bacteria everywhere?

Detection of ESBL-carrying multi-resistance plasmids in clinical *Escherichia coli* isolates from food-producing animals

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Objective: The aim of this study was to investigate the co-location of antimicrobial resistance genes on extended-spectrum β-lactamase (ESBL) gene-carrying plasmids in *Escherichia coli* isolates from diseased food-producing animals.

Methods: A total of 3670 *E. coli* isolates was collected from diseased animals in the German National Resistance Monitoring program GE*RM*-Vet during 2010-2013. In 140/1783 bovine, 46/819 porcine and 8/1068 avian isolates the presence of ESBL genes was confirmed by PCR and sequencing. Representative isolates (n=50) and their plasmids were characterized by susceptibility testing to 28 antimicrobial agents, XbaI-macrorestriction analysis, multilocus sequencing typing (MLST), phylotyping, electrotransformation and conjugation experiments, replicon typing, S1 nuclease PFGE and PCR assays for the detection of resistance genes.

Results: The 50 ESBL-producing *E. coli* isolates displayed 48 unrelated and two closely related XbaI-macrorestriction patterns and 23 MLST types [most common ST10 (n=5), ST167 (n=8) and ST362 (n=5)]. The isolates were distributed among the phylogenetic groups A (n=27), B1 (n=9), B2 (n=1) and D (n=13). Transfer experiments revealed the presence of single ESBL genes on the plasmids [$bla_{CTX\text{-}M\text{-}1}$ (n=24), $bla_{CTX\text{-}M\text{-}14}$ (n=17), $bla_{CTX\text{-}M\text{-}15}$ (n=4), $bla_{CTX\text{-}M\text{-}3}$ (n=1) and $bla_{SHV\text{-}12}$ (n=4)], with 41/50 plasmids being conjugative. Fifteen ESBL-carrying plasmids harboured solely β-lactam resistance genes, while another twelve plasmids harboured genes conferring resistance to a second class of antimicrobial agents, e.g. aminoglycosides [*aac(3)-IIa* and *aac(3)-IVa*] or phenicols (*floR*), or conferring reduced susceptibility to fluoroquinolones (*qnrS1*). Multi-resistance (resistance to at least three classes of antimicrobial agents) was identified in 23 plasmids, including seven $bla_{CTX\text{-}M\text{-}1}$-, nine $bla_{CTX\text{-}M\text{-}14}$-, four $bla_{CTX\text{-}M\text{-}15}$-, and three $bla_{SHV\text{-}12}$-carrying plasmids, with sizes of 30-330 kb. These plasmids carried, most commonly, genes for resistance to sulphonamides (*sul1* or *sul2* or *sul3*), trimethoprim (*dfrA* variants), or tetracycline [*tet*(A) or *tet*(B)] and 16/23 were conjugative. The multi-resistance plasmids encoding CTX-M-1 belonged to the incompatibility groups IncI1 (n=2), IncF (n=1), FIA+FIB (n=2), IncHI2 (n=1), or IncX (n=1), all coding for CTX-M-14 to IncF, for CTX-M-15 to IncF+FIA+FIB (n=2), IncI1 (n=1) or IncN (n=1), and for SHV-12 to IncI1 (n=2) or IncF (n=1). The isolates that carried these multi-resistance plasmids belonged to various MLST types, with ST10 (n?=?4) and ST167 (n?=?4) being the most common, showed unrelated or two closely related XbaI-macrorestriction patterns and belonged to the phylogenetic group A (n=16), B1 (n=2) or D (n=5).

Conclusions: The presence of additional resistance genes on the ESBL-carrying plasmids suggests that co-selection of ESBL genes may occur even in the absence of β-lactam antibiotics and may lead to the presence of ESBL producers in animals and humans. Moreover, the identification of conjugative multi-resistance ESBL-carrying plasmids in *E. coli* isolates from food-producing animals underlines the risks of resistance dissemination to humans as such isolates may enter the food chain.