Analysis of *bla*$_{\text{CTX-M-1}}$-carrying plasmids from *Escherichia coli* isolates collected in the BfT-GermVet study in Germany

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The aims of this study were to determine the prevalence of extended spectrum β-lactamase (ESBL) genes in *Escherichia coli* from defined disease conditions of companion and farm animals from the BfT-GermVet study and to gain insight into the localization and organization of the ESBL gene regions.

In the BfT-GermVet study, 417 *E. coli* isolates from diseased dogs/cats (n = 228), horses (n = 102), and swine (n = 87) were tested for their susceptibility to 24 antimicrobial agents by broth microdilution. To identify potential producers of extended spectrum β-lactamases (ESBLs), all 100 ampicillin-resistant *E. coli* isolates were subjected to an initial screening for cefotaxime resistance and subsequent phenotypic confirmatory tests. The ESBL genes were detected by PCR and the ESBL gene regions were cloned and sequenced. Plasmid transfer experiments included conjugation and transformation into *E. coli* recipients and the plasmids were typed by PCR-based replicon typing. Multilocus sequence typing was performed for the ESBL producing *E. coli* isolates.

Two of the 100 ampicillin-resistant *E. coli* isolates, one from canine pneumonia (isolate168) and the other from porcine mastitis-metritis-agalactia syndrome (isolate 246), showed an ESBL phenotype. Multilocus sequence typing confirmed that each of them belonged to a novel sequence type, namely ST1153 (isolate 246) and ST1576 (isolate 168). ESBL genes of the type *bla*$_{\text{CTX-M-1}}$ were detected in both isolates. They were located on structurally related plasmids of ca. 50kb which did not confer any other resistance properties. PCR-based replicon typing identified both plasmids, designated pCTX168 and pCTX246, as being positive for IncN, repFIA, repFIB, repFIC and IncF. They showed the following genetic organization of the *bla*$_{\text{CTX-M-1}}$ gene area: In the upstream region, a fragment of the insertion sequence IS*Ecp1*, truncated by an IS*26* element, was detected whereas in the downstream region, a truncated mrx gene was identified. On plasmid pCTX246, a *mph*(A) gene and another IS*26* element were seen further downstream of the Δ*mrx* gene, while on plasmid pCTX168 the *mph*(A) gene was truncated by the integration of a segment homogenous to a ca. 56-kb plasmid from *E. coli*.

The gene *bla*$_{\text{CTX-M-1}}$ in combination with the IS*26*-ΔIS*Ecp1*-structure on IncN plasmids has been recently decribed in a German human clinical *E. coli* isolate but not in isolates of animal origin in Germany so far. IncN plasmids, carrying *bla*$_{\text{CTX-M-1}}$, have been described to be transmitted between Danish farm personnel and pigs in different *E. coli* lineages, suggesting an animal reservoir for this ESBL gene variant. The results of this study showed that the integration of insertion sequences and interplasmid recombination events account for the structural variability of the *bla*$_{\text{CTX-M-1}}$ region.