Objective: Characterisation of 17 clinical E. coli isolates producing different types of chromosomally encoded CTX-M-enzymes. The isolates were collected from The Netherlands, United Kingdom and Germany within the EU SAFEFOODERA project “The role of commensal microflora of animals in the transmission of extended spectrum β-lactamases”. Methods: The identification of Beta-lactamases was performed by PCR/sequencing. The isolates were typed by MLST, phylogenetic groups and XbaI-PFGE. The plasmid content was visualized by S1-nuclease-PFGE. The blaCTX-M-genes were mapped by Southern-blot hybridization using XbaI- and S1-PFGE profiles. To confirm the chromosomal location of the blaCTX-M-genes, the genomic DNA was also digested with I-CeuI, and I-CeuI-PFGE profiles were hybridized with blaCTX-M- and16S rDNA-probes.

Results: The 17 clinical E. coli were mostly ascribed to the phylogenetic groups D (n= 9) and B2 (n= 6). Only two isolates belonged to group B1. Both were CTX-M-15-producers but showed different sequence types (ST156 and ST2178). Among all 17 isolates seven different STs were identified and one had not been described before. The most predominant was ST38, found in 7 isolates which belonged to phylotype D. Most isolates (n=11) produced CTX-M-15, mainly in addition to TEM-1 (n= 5), OXA-1 (n= 2) or both (n= 2). The remaining 6 isolates expressed CTX-M-group 9 Beta-lactamases, being CTX-M-14 the most frequent one (4 isolates, 2 of them co-produced TEM-1). CTX-M-9 and CTX-M-51 were detected only in one isolate each. Hybridization experiments confirmed, that CTX-M-encoding genes were all chromosomally located. Positive hybridization bands were only detected in XbaI- (ranging from ~95-600 kb) and I-CeuI-PFGE (bands of ~550-1080 kb) profiles. Two strains showed the presence of two copies of the blaCTX-M gene in their chromosome. Plasmids in contrast turned out to be negative in hybridization experiments. Conclusion: The presence of these epidemiologically relevant antimicrobial resistance genes on the chromosome, rather than on plasmids promotes the maintenance of the gene in the population before their dissemination.