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The genetic diversity of commensal *Escherichia coli* strains isolated from non-antimicrobial treated pigs varies according to age

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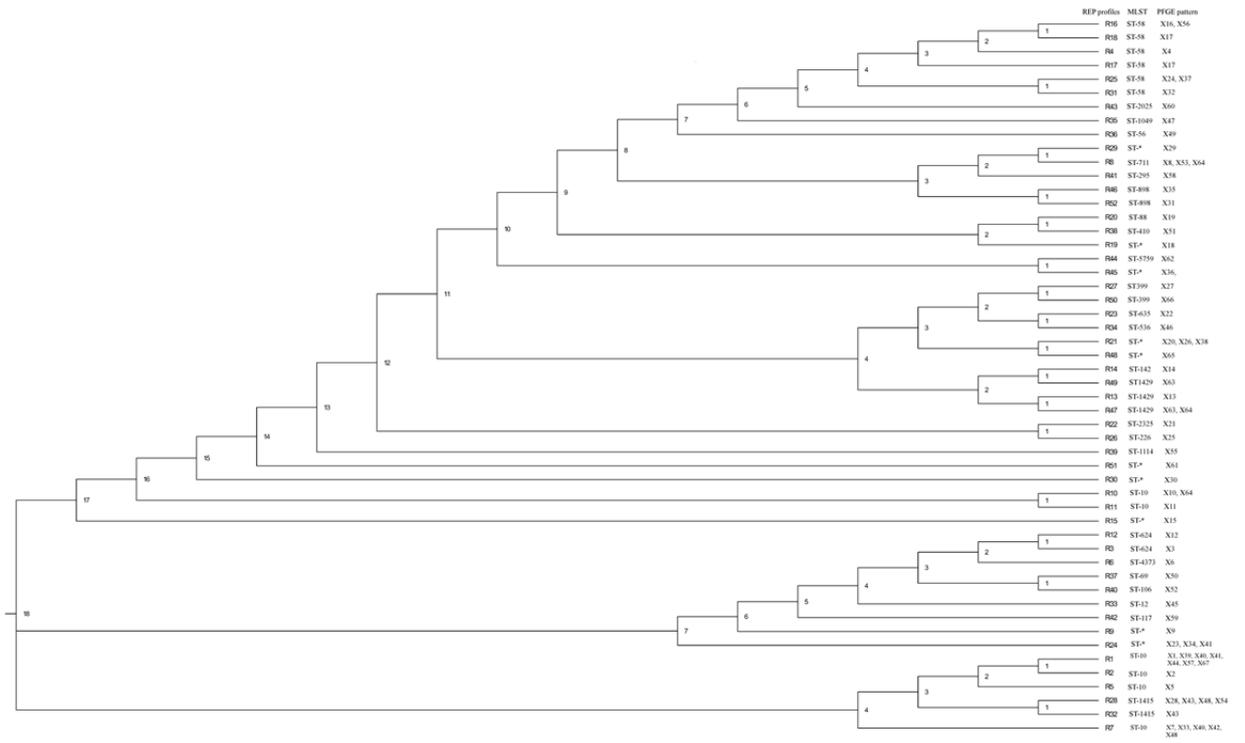
Background: This study investigates the genetic diversity and relationship of *E. coli* strains obtained from fecal samples from non-antimicrobial treated pigs belonging to five different age groups from a Danish farm. This is the first report on the genetic diversity of commensal *E. coli* from guts of pigs reared in an antibiotic free production system.

Material/methods: 900 colonies were isolated from fecal samples collected from 20 pigs (N=4 pigs/age group: piglets, early weaners, late weaners, finishers and sows) and confirmed to be *E. coli* by biochemical characterization. Diversity was assessed by using repetitive extra-genic palindromic-PCR (REP-PCR), followed by pulsed field gel electrophoresis (PFGE) and whole genome sequence (WGS) analysis. The Shannon diversity index (H') was used to calculate the genetic diversity. Bionumerics was used to analyze the relatedness among the REP and PFGE fingerprints obtained, and similarities were calculated using Pearson coefficient. Dendrograms were obtained using UPGMA.

Results: A total of 52 unique REP profiles were detected. At the pig level, the number of different strains ranged from 2 to 13 per pig. The dominant profiles, R1, R7 and R28, were the most frequently detected in all age groups. Among the age groups, the highest and the lowest degree of diversity were found in the early weaners (H' of 2.215) and piglets (H' of 1.461), respectively. *E. coli* isolates yielding unique REP profiles and several strains assigned to the most frequent profiles were subjected to PFGE and yielded 67 different genotypes. The genetic relationship of REP-profiles within each age group, between groups as well as between the different PFGE genotypes was also investigated. WGS analysis of the 52 isolates assigned to unique REP profiles showed a great genotype variation among them. SNP phylogeny was also established (Figure).

Conclusions: A high degree of diversity was observed between *E. coli* isolates within all the age groups tested and between the groups. The dominant strains (assigned to profiles R1, R7 and R28) are suggested to represent archetypal commensal *E. coli* circulating among Danish pigs since they were also the most abundant in previous studies on *E. coli* diversity in antibiotic treated pigs. Despite the strains were isolated from non-treated healthy pigs, the multiple resistance-gene rate among the 52 sequenced isolates was 15,4% and some of the strains might be potential pathogenic according to their virulence pattern, serotype and ST type. Studies on genetic diversity might contribute to better characterize the commensal niche and to increase knowledge on the population genetics and their spread. They may also allow implementing more accurate modeling studies with different purposes, such as studies on emergence and selection of antimicrobial resistance.

Figure. SNP phylogeny of *E. coli* representing 52 unique REP profiles.
ST-*, unknown sequence type



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