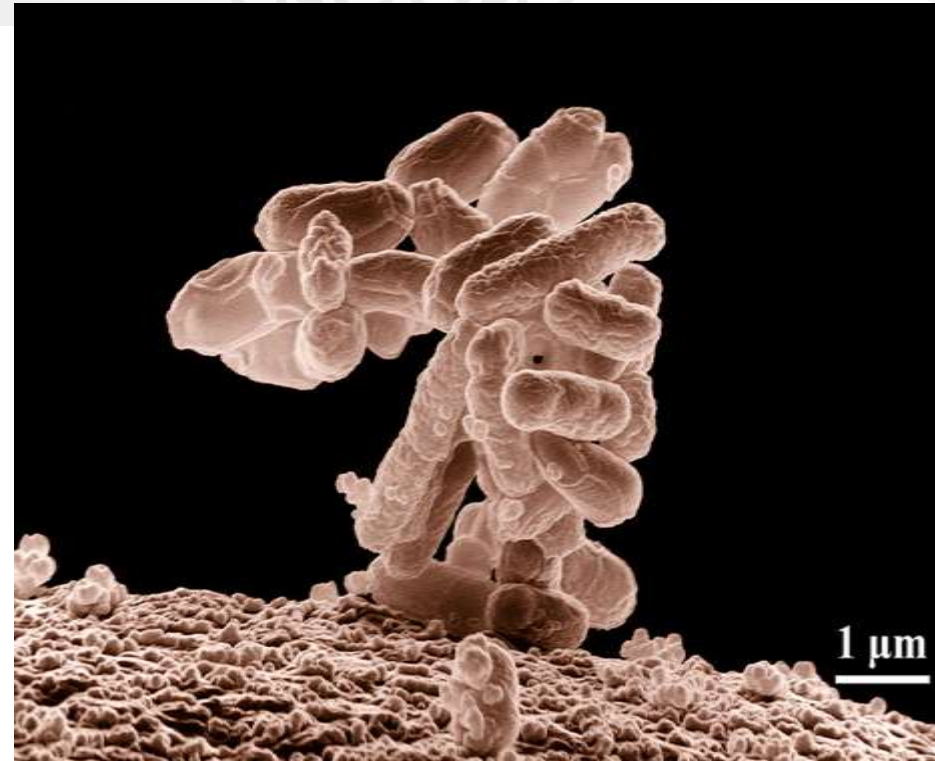


The genetic diversity of commensal *Escherichia coli* strains isolated from non-antimicrobial treated pigs varies according to age group

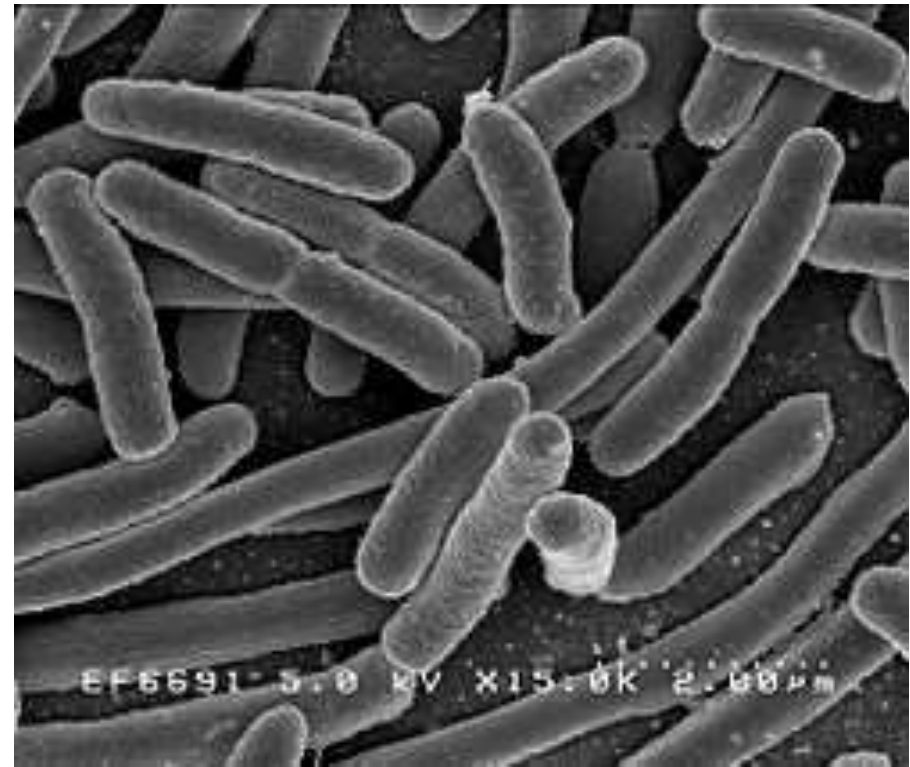


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Introduction

- *E. coli*; a gram-negative, rod-shaped bacterium
- Human and animal guts are the primary habitat of *E. coli*
- "Indicator" bacteria



Objectives

Goals?

To determine the genetic diversity and relationship of *E. coli* commensal strains isolated from non-antimicrobial treated pigs belonging to different age groups

To characterize the strains assigned to unique REP-profiles

How?

Molecular typing methods:
-REP-PCR
-PFGE
-Statistical approaches (H', phylogenetic trees)

WGS and CGE
(www.genomicepidemiology.org/)

Study design

20 fecal samples (20 pigs) from 5 age groups
Piglets (N=4), Early weaners (N=4), Late weaners (N=4),
Finishers (N=4) and Sows (N=4)

900 *E. coli* (180/pig feces) isolates analyzed by REP-PCR

PFGE: 76 str assigned to different
REP profiles (at least one
representative of each unique
REP)

WGS: 52 str assigned to unique
REP-Profiles

First report on genetic diversity of commensal *E. coli* from pigs in an Ab-free production system and belonging to 5 different age groups

Age groups	No. of isolates	No. of unique REP profiles	H'
Piglets	180	17	1.46
Early weaners	180	24	2.22
Late weaners	180	21	1.91
Finishers	180	13	1.61
Sows	180	17	1.49
Total	900	52	2.05

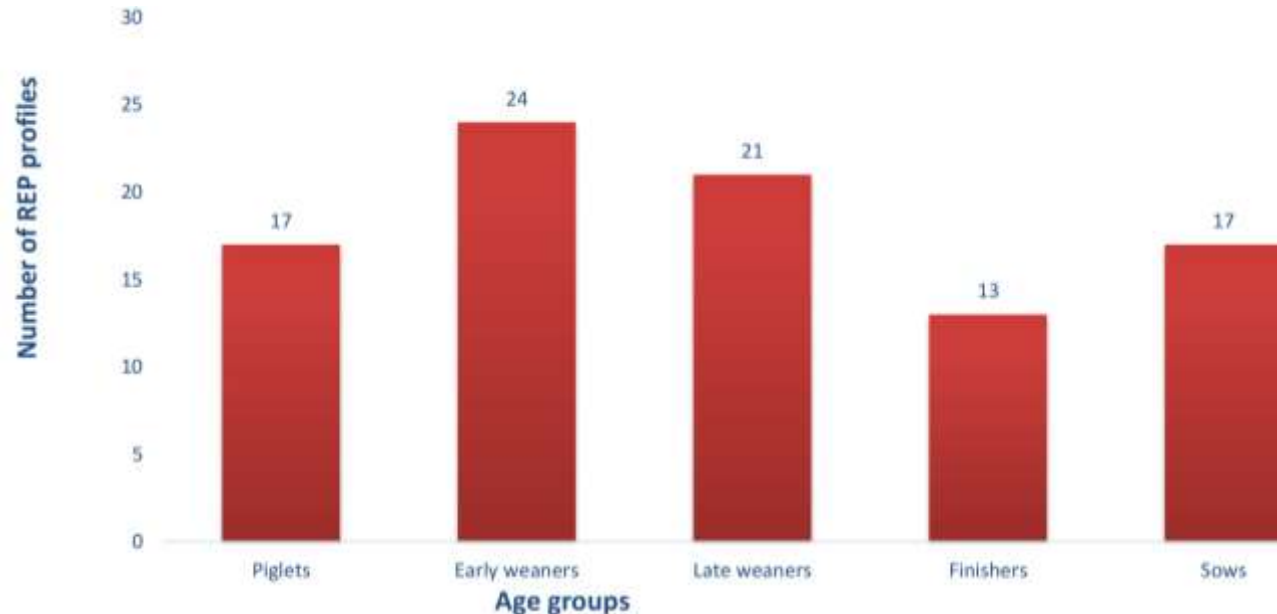


Fig and Table. Frequency and distribution of different *E. coli* strains (REP profiles) within each age group of pigs.

52 unique REP profiles were detected suggesting a high degree of diversity. The number of str. per pig ranged from two to 13. Highest and lowest degree of diversity were found in early weaners and piglets.

The REP profiles, R1, R7 and R28, were the most frequently observed in all age groups

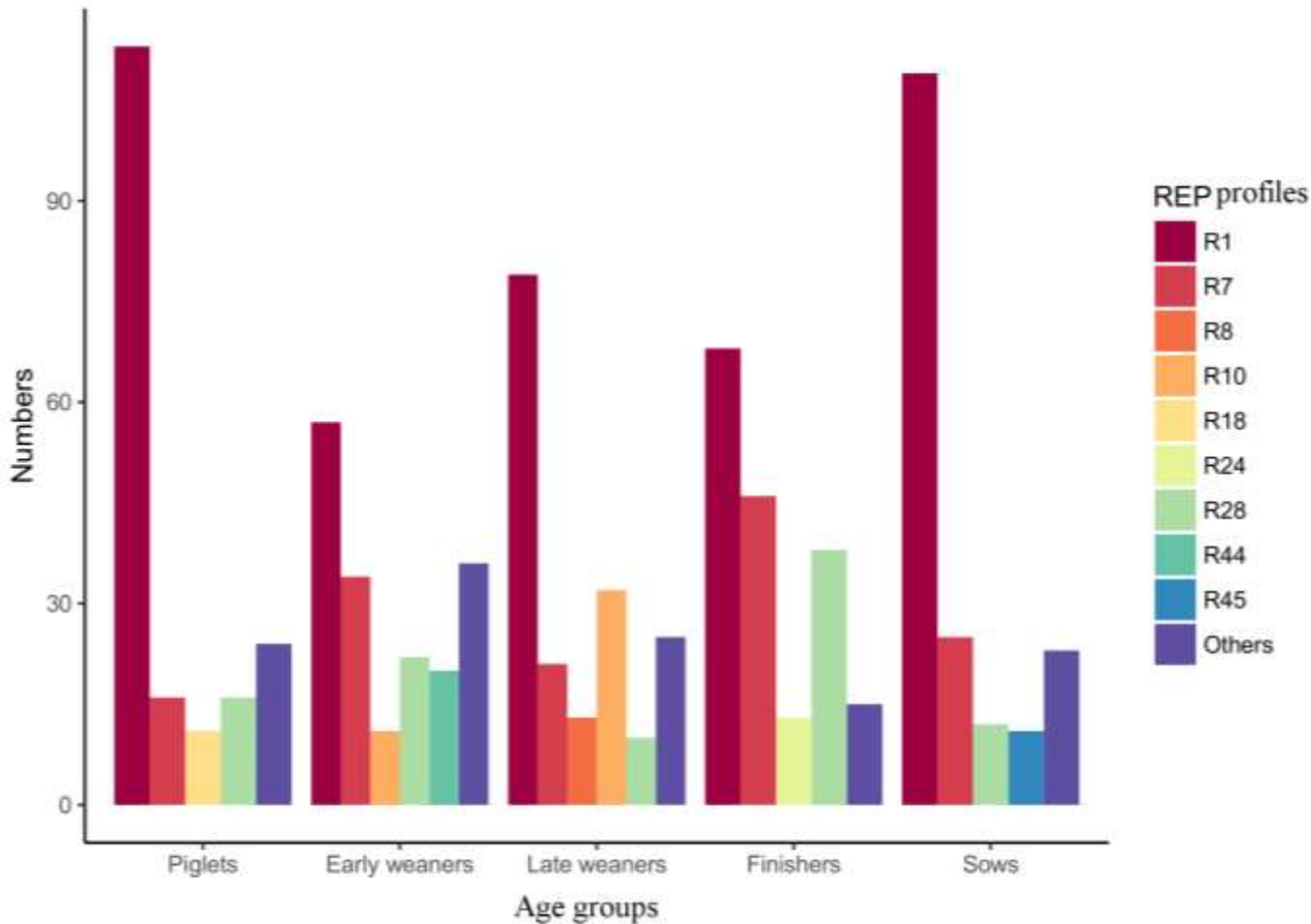


Fig. Distribution of REP profiles among the five age groups of pigs. Dominant REP profiles represented by at least 10 strains are shown. The sum corresponding to the rest of the REP profiles detected in each age group is termed as “Others”.

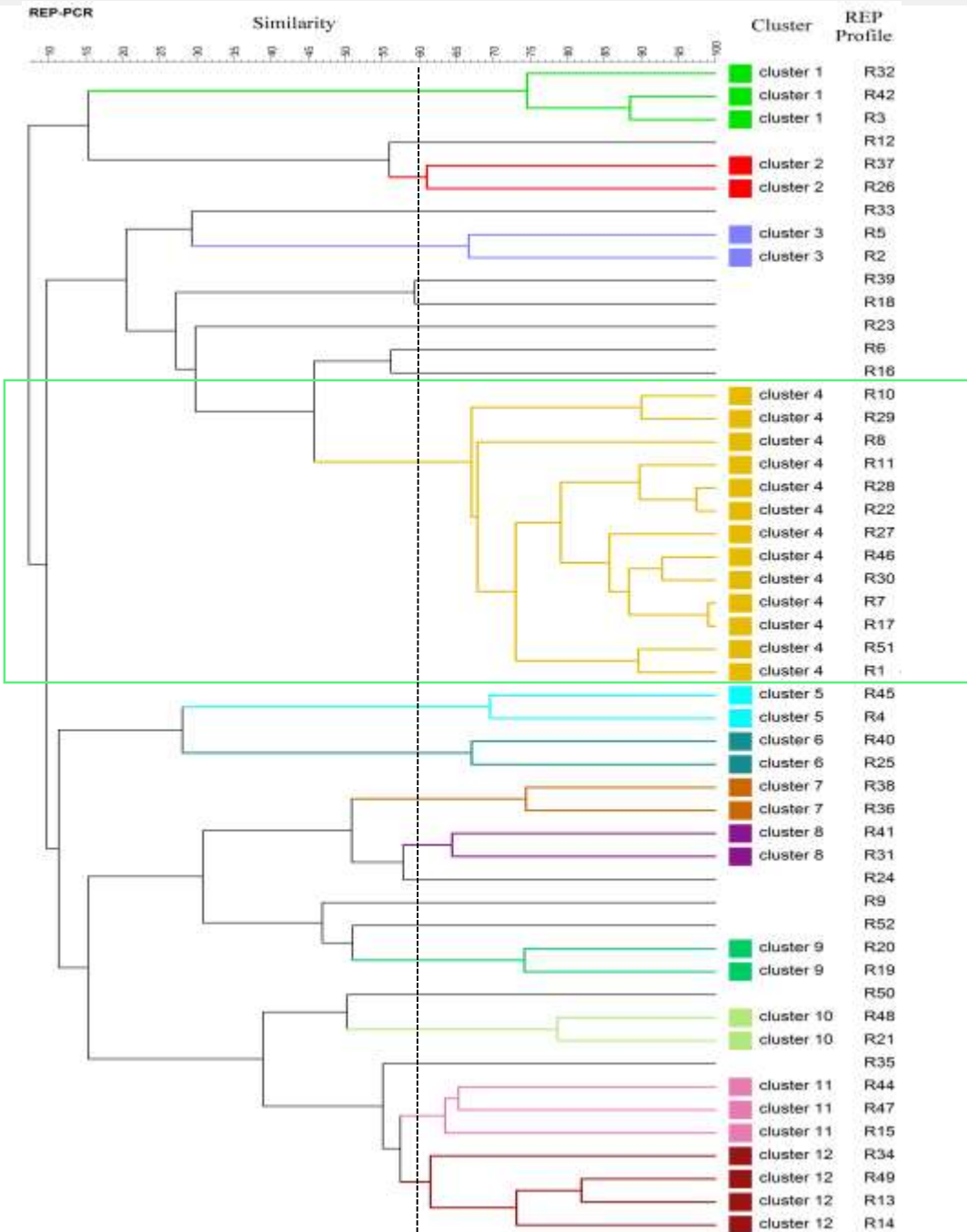


Fig 2. Dendrogram showing the relatedness of all REP-PCR fingerprints generated from *E. coli* strains isolated from all pigs included in the study. Pearson coefficient was used to calculate REP profiles similarities and the dendrogram was generated by UPGMA.

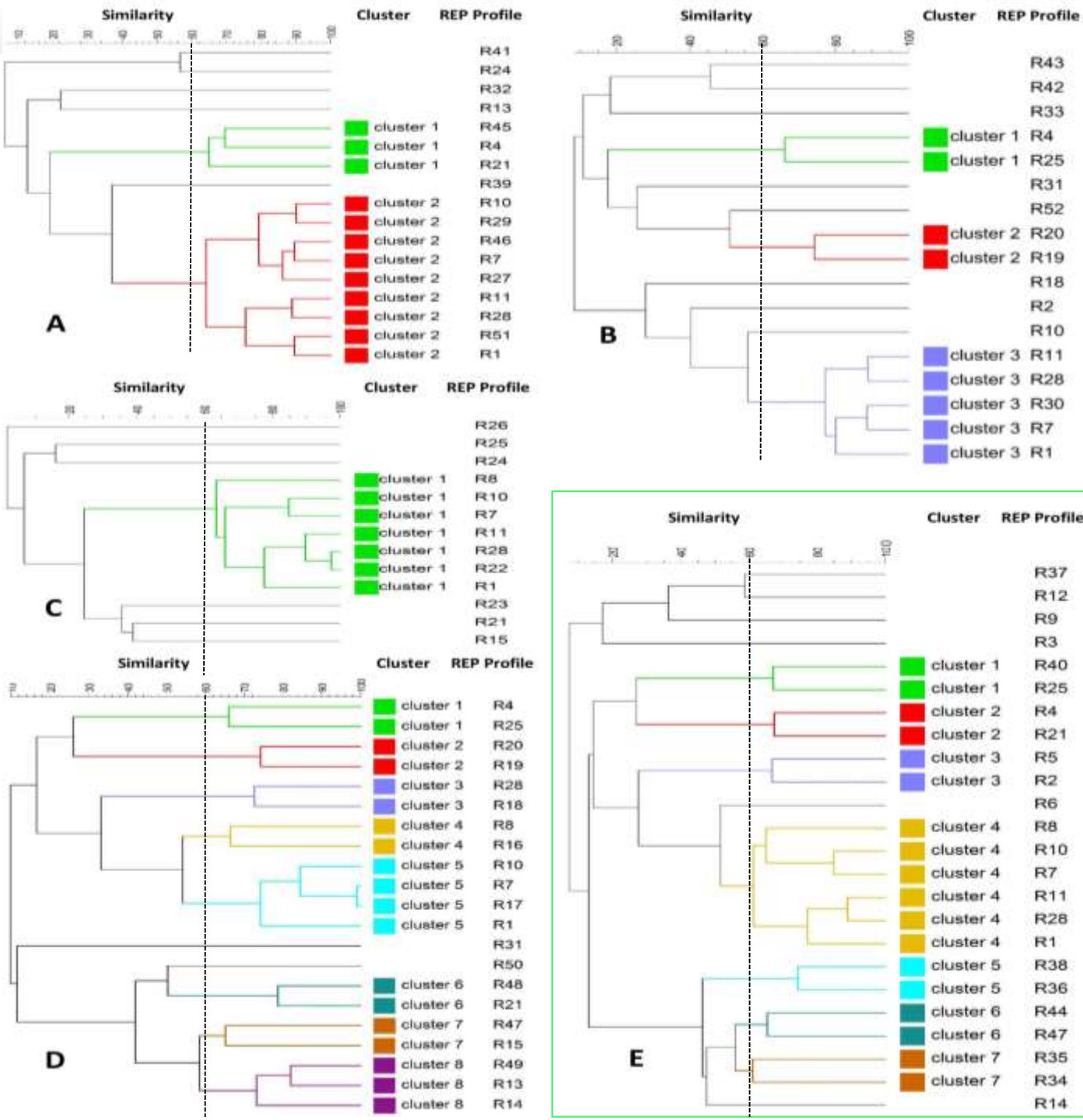
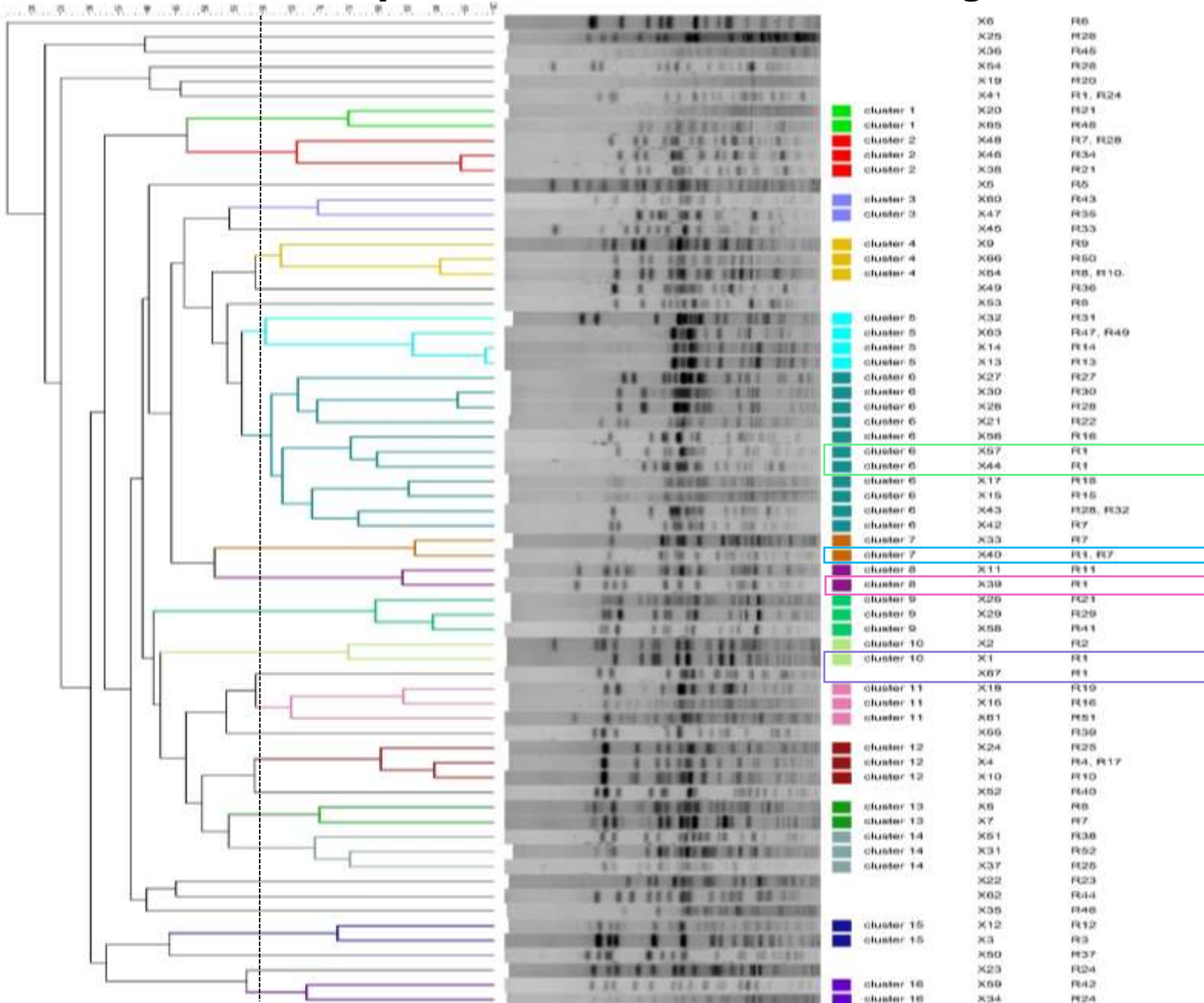


Fig 3. Dendrogram showing the relatedness of REP-PCR fingerprints of *E. coli* strains within each age group of pigs including sows (A), piglets (B), finishers (C), late weaners (D) and early weaners (E). Pearson coefficient was used to calculate REP profiles similarities and the dendrogram was generated by UPGMA

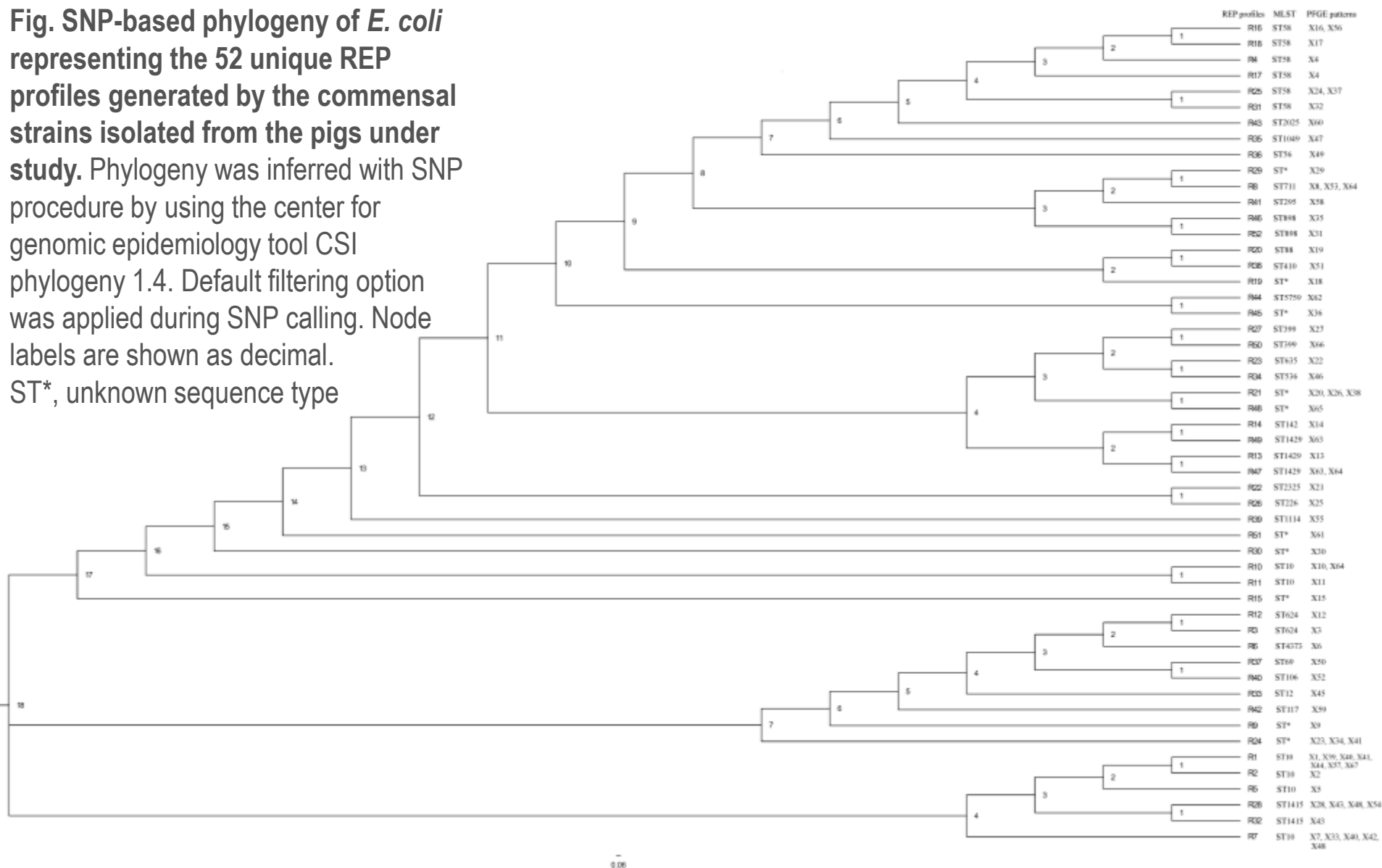
67 PFGE patterns were identified among 76 isolates



Whole Genome sequence analysis-CGE

- *52 str assigned to unique REP profiles subjected to WGS*
- ✓ **MLST type:** 25 ST types in 42 str, 10 unknown ST. Dominant: ST10 (11.5 %) and ST58 (11.5%).
- ✓ **Serotype:** 37 serotypes. Dominant O19:H7 (11.5 % O8:H19 (7.7%) and O8:H10 (7.7 %).
- ✓ **Antimicrobial R-genes:** Ap (21.1 %), Str (21.1 %), Sul (13.5 %), Tp (17.3%), Tet (5.8%), Eri (3.8%), Chl (1.9 %), Kn (1.9%)
- ✓ **Plasmids:** 29 types of replicons (96%)
- ✓ **V- genes:** 21 types, at least one in 51 isolates out of 52
- ✓ **SNP tree;** N of diff. SNPs; from 1 (R10 and R11) to 39789 (R33 and R45).

Fig. SNP-based phylogeny of *E. coli* representing the 52 unique REP profiles generated by the commensal strains isolated from the pigs under study. Phylogeny was inferred with SNP procedure by using the center for genomic epidemiology tool CSI phylogeny 1.4. Default filtering option was applied during SNP calling. Node labels are shown as decimal. ST*, unknown sequence type



Isolates	REP	R-genes	V- genes	MLST	Plasmids	Serotype	Acc. Number
P1.08	R1	-	astA, gad, iss	ST10	-	O:H11	ERS1363800
P1.20	R2	mph(A)-like	astA, celb, iss	ST10	IncFII, Col(BS512), Col8282, ColRNAI, Col156, Col(MG828)	O13:H11	ERS1363801
P1.49	R3	sul2	air, gad	ST624	ColRNAI	O:H1	ERS1363802
P1.51	R4	strB	iss, lpfA	ST58	ColRNAI	O8:H10	ERS1363803
P1.53	R5	bla _{TEM-1B} , sul2	astA, gad, iss	ST10	IncFII, ColRNAI	O:H11	ERS1363804
P1.56	R6	aadA1, bla _{TEM-1B} , catA1-like, dfrA1, strA, strB, sul2, tet(B)	air, astA, gad, iss, lpfA	ST4373	IncQ1	O:H34	ERS1363805
P1.57	R7	aadA1, bla _{TEM-1C} -like, mph(A)	iss	ST10	IncFII, IncX1	O:H19	ERS1363806
P1.69	R8	bla _{TEM-1B} -like, tet(A)	astA, celb, lpfA	ST711	IncX1, Col(MG828), Col156, ColRNAI	O:H25	ERS1363807
P1.75	R9	aadA1, strB	gad, ireA, iroN, iss, lpfA, pic	Unknown ST	IncFIB(AP001918), IncI1, IncFIC(FII), ColRNAI, Col(MG828)	O8:H4	ERS1363808
P1.77	R10	-	gad	ST10	IncHI2A, IncHI2, IncX1, ColRNAI	O109:H27	ERS1363809
P1.78	R11	-	gad	ST10	IncHI2A, IncFIA, IncHI2, IncX1, ColRNAI	O109:H27	ERS1363810
P1.85	R12	sul2	air, eilA, gad, lpfA	ST624	ColRNAI	O:H1	ERS1363811
P1.99	R13	-	celb, gad, lpfA	ST1429	IncP(6), IncX1, Col156, ColRNAI	O19:H7	ERS1363812
P1.101	R14	-	celb, gad, lpfA	ST1429	IncX1, IncP(6), Col156, ColRNAI	O19:H7	ERS1363813
P1.115	R15	aadA1, dfrA5, strA, strB-like	astA, ccl, iroN, lpfA	Unknown ST	IncFIB(K), IncFII, IncFIB(AP001918), IncFII(pCRY), Col(MG828), ColRNAI	O9:H19	ERS1363814

Isolates	REP	R-genes	V- genes	MLST	Plasmids	Serotype	Acc. Number
P1.123	R16	dfrA5	cba, gad, iss, lpfA, mchF	ST58	IncFII, IncI1, IncFIB(AP001918), ColRNAI	O8:H10	ERS1363815
P1.124	R17	bla _{TEM-1B} -like, dfrA5	cba, cma, gad, iss, lpfA, mchF	ST58	IncFII, IncI1, IncFIB(AP001918), ColRNAI	O8:H10	ERS1363816
P1.127	R18	dfrA5, strA, sul2	cba, cma, gad, iss, lpfA, mchF	ST58	IncFII, IncI1, IncFIB(AP001918), ColRNAI	O8:H10	ERS1363817
P1.134	R19	bla _{TEM-1B} , dfrA5	iroN, iss, lpfA, mchF	Unknown ST	IncFIB(AP001918), IncX1	O21:H21	ERS1363818
P1.135	R20	dfrA5, strA, strB, sul2	gad, iss, lpfA, mchF	ST88	IncFIB(AP001918)	O8:H19	ERS1363819
P1.154	R21	-	gad, lpfA	Unknown ST	IncY	O19:H7	ERS1363820
P1.173	R22	-	iss	ST2325	-	O66:H25	ERS1363821
P1.176	R23	-	gad, lpfA	ST635	ColRNAI	O11:H25	ERS1363822
P1.187	R24	-	ireA, iss, lpfA, mchB, mchC, mchF	Unknown ST	IncFIC(FII), IncFIB(AP001918), ColRNAI	O127:H4	ERS1363823
P2.08	R25	aph(3')-Ia-like, bla _{TEM-1B} , dfrA5, strB-like	gad, iroN, iss, lpfA	ST58	IncFII, IncFIB(AP001918), ColRNAI	O8:H19	ERS1363824
P1.192	R26	-	gad	ST216	IncFIA(HI1), IncHI1A, IncHI1B(R27), IncFIB(K), p0111	O3:H4	ERS1363825
P1.204	R27	-	gad, lpfA	ST399	IncY, ColRNAI	O13/O135:H3	ERS1363826
P2.200	R28	-	astA, iss	ST1415	IncFII	O108:H34	ERS1363827

Isolates	REP	R-genes	V- genes	MLST	Plasmids	Serotype	Acc. Number
P1.238	R29	-	astA, gad, iss, lpfA	Unknown ST	IncFIC(FII), IncFIB(AP001918), ColRNAI	O70:H10	ERS1363828
P2.02	R30	-	astA, iss, lpfA	Unknown ST	IncFIB(AP001918), ColRNAI	O60:H5	ERS1363829
P2.19	R31	bla _{TEM-1B} , dfrA5, strA, strB-like, sul2-like	iroN, iss, lpfA, mchF	ST58	IncFII, IncFIB(AP001918), ColRNAI	O8:H19	ERS1363830
P2.194	R32	-	astA, gad, iss	ST1415	IncFII	O108:H34	ERS1363831
P3.01	R33	-	cnf1, gad, iroN, iss, mchB, mchC, mchF, mcmA, vat	ST12	ColRNAI	O4:H5	ERS1363832
P3.52	R34	-	gad, lpfA	ST536	IncY	O154:H9	ERS1363833
P3.62	R35	-	gad, lpfA	ST1049	ColRNAI	O32:H10	ERS1363834
P3.69	R36	bla _{TEM-1B} , tet(A)	iroN, iss, lpfA, mchF	ST56	IncFIB(AP001918), IncX1	O21:H21	ERS1363835
P3.71	R37	bla _{TEM-1B}	air, eilA, gad, iss, lpfA, mchB, mchC, mchF, mcmA, tsh	ST69	IncFIC(FII), IncFII(29), IncFIB(AP001918)	O15:H6	ERS1363836
P3.75	R38	-	iss, lpfA	ST410	IncFIB(AP001918), Col(MG828), ColRNAI	O:H9	ERS1363837
P3.203	R39	-	gad	ST1114	Col8282, ColRNAI	O98:H26	ERS1363838
P3.96	R40	-	air, eilA, gad, iss, lpfA	ST106	IncFII	O17/O44:H18	ERS1363839
P3.235	R41	-	astA, gad, lpfA	ST295	IncFII, IncFIB(AP001918)	O171:H21	ERS1363840

Isolates	REP	R-genes	V- genes	MLST	Plasmids	Serotype	Acc. Number
P4.25	R42	-	gad, ireA, iss, lpfA, mchB, mchC, mchF, vat	ST117	ColRNAI	O114:H4	ERS1363841
P4.31	R43	-	gad, iroN, iss, lpfA	ST2025	ColRNAI	O8:H25	ERS1363842
P4.53	R44	-	air, astA, eilA, iss, stb	ST5759	IncFII(29), IncFIB(AP001918)	O:H20	ERS1363843
P4.194	R45	-	gad	Unknown ST	Col8282, Col156, ColpVC, Col(MG828)	O:H7	ERS1363844
P4.200	R46	aadA1, tet(B)	lpfA	ST898	Col(MG828)	O154:H48	ERS1363845
P4.88	R47	-	celb, gad	ST1429	IncP(6), IncX1, Col156, ColRNAI	O19:H7	ERS1363846
P4.132	R48	-	gad, lpfA	Unknown ST	Col(MGD2), ColRNAI, IncY, IncX1, IncL/M(pOXA-48), Col(Ye4449)	O19:H7	ERS1363847
P4.136	R49	-	celb, lpfA	ST1429	IncX1, IncP(6), Col156, ColRNAI	O19:H7	ERS1363848
P4.143	R50	-	gad, lpfA	ST399	IncY	O13/O135: H30	ERS1363849
P4.233	R51	-	-	Unknown ST	IncFII(29), Col8282, ColE10, Col156, ColpVC, Col(MG828)	O142:H38	ERS1363850
P2.09	R52	aadA1, bla _{TEM-1B} -like, dfrA1	iss, lpfA	ST898	IncY, ColRNAI	O:H48	ERS1363851

Conclusions

1. **High degree of diversity within and between age groups of pigs** in the absence of Ab-treatment. A single pig = 1 predominant str. of *E. coli* + 1 to few other str.
2. **Ab-treatment**; not as important in determining genetic diversity of the *E. coli* commensal microbiota as assumed: diversities in early weaners similar to those observed in Ab-treated nursery pigs (previous studies).
3. **Age**; Genetic diversity in *E. coli*, (although no significant differences) varied depending on age group with *E. coli* from early weaners showing highest diversity; age **could be a contributing factor to the genetic diversity** observed among *E. coli*.
4. As in previous studies, profiles; **R1, R7 and R28 were dominant**. The str assigned to these profiles may represent the archetypal commensal *E. coli*. The three strains (associated with R1, R7 and R28) sequenced in the current study belonged to the sequence type ST10 (R1 and R7), and ST1415 (R28).
5. Different REP profiles led to same PFGE pattern and the other way around, thus, there is **not a correlation between REP and PFGE patterns**. The SNP phylogenetic tree revealed that **PFGE typing had a better discriminatory power**; in most of cases, similar PFGE patterns clustered together in the SNP tree too (also detected for same ST types).

Conclusions

- 6. None of the 52 isolates harbored ESBL genes** (3rd and 4th generation cephalosporins not used in the pig production in Denmark). Prevalence of **one-antimicrobial-R gene carriage and 3 or more R-genes were 40.4 and 15.4 %** . Most abundant R-genes found also detected in *E. coli* from pigs in Denmark, USA and France.
- 7. The 52 E. coli isolates harbored broad range of V-genes.** 7 str contained at least one adhesion factor and one toxin gene; **13% of randomly collected commensal E. coli could be potentially pathogenic strains, despite samples collected from healthy animals.** Three of these 7 isolates harbored multiple R-genes, and six of them carried the gene for long polar fimbriae (lpfA) V-gene, described as a potential-V marker for pathogenic *E. coli*.
- 8. The dominant ST types were ST10 (six strains out of 52- also dominant in previous study) and ST58 (six strains)** which contrary to our observation, are most often reported as ESBL producing *E. coli* (also commonly found in chickens, other animals and humans). 1 str assigned to ST117, an APEC *E. coli* with zoonotic potential.
- 9. The dominant serotypes were O19:H7 (6 isolates) followed by O8:H19 (4 isolates) and O8:H10 (4 isolates).** Most frequent O antigen was O8 (9 strains) -commonly reported antigen associated with porcine pathogenic *E. coli*- O108 (2 strains), usually linked to porcine pathogenic isolates.
- 10. 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 accurate modeling studies with different purposes

Thank you for your attention!

