Effect of usage / non-usage of antibiotics on virulence profiles of *Escherichia coli* in pig production.

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Introduction

Escherichia coli are responsible for acute profuse diarrhoea in growing pigs with resultant high morbidity and mortality (Toledo *et al.,* 2012). Diarrheagenic *E. coli* pathovars in pig enteritis are enterotoxigenic *E. coli* (ETEC) encoding heat stable (STa, STb, EAST1) and/or heat labile (LT) enterotoxins (Gyles, 2010), while shiga toxin *E. coli* (STEC) strains encode the Shiga toxin type 2e (Stx2e) and cause edema disease. Porcine ETEC and STEC strains have plasmid-mediated surface fimbrial structures (Dubreuil et al., 2016), which enable the bacteria to colonize the epithelial surface of the pig small intestine. These include but not limited to: F4 (K88), F5 (K99), F6 (P987), F18 and F41 (Frydendahl, 2002). Antibiotics are frequently used in the treatment and control of these enteric infections in pigs but long term administration, misuse and abuse may lead to unintended consequences.

Methodology

In a controlled study, two groups of growing pigs (5 piglets per group) under routine farm management practices were identified and classified as antibiotics (ATG) and non-antibiotic groups (NAG) based on antibiotics administration. Rectal swabs were taken from all piglets on days 1, 5, 10, 21, 28, 35, 56 and 70. *E.coli* were isolated from the swabs, DNAs were extracted and used for further PCR amplification using the boiling method. Metagenomic analysis was carried out to classify the bacteria populations in relations to antibiotics usage

Results

A total of 241 *E.coli* strains were isolated from both groups between days 0 and 70. Virulence genes were detected by PCR in 24.8% (18.2 - 32.7) of the ATG isolates and 43.5% (34.5 - 52.9) of the NAG with a significant difference (P = 0.002). AIDA1 was the most dominant non-fimbrial adhesion factor while F6 was the only fimbrial factor detected. Twelve pathotypes were identified, with pathotype EAST1 being the most prevalent.

Table 2. Pathotype of isolates that harboured virulence genes

Pathotypes	ATG (n=33)	NAG (n=47)	P-value
EAST1	60.6 (43.64-75.32)	61.7 (47.43-74.21)	0.92
STa	18.1(8.61-34.39)	0 (0-7.55)	**<0.005**
STa/F6	0 (0.0-10.43)	2.1 (0.37-11.11)	0.4
STb/EAST1/AIDA	0 (0.0-10.43)	8.5 (3.36-19.93)	0.09
Stx2e	3.0 (0.53-15.32)	0 (0-7.55)	0.23
EAST1/EAE	0 (0.0-10.43)	2.1 (0.37-11.11)	0.4
EAST1/PAA	15.1 (6.65-30.92)	0 (0-7.55)	**<0.01**
EAST1/AIDA1	3.0 (0.53-15.32)	8.5 (3.36-19.93)	0.32
EAST1/STa	0 (0.0-10.43)	2.1 (0.37-11.11)	0.4
EAST1/STa/F6	0 (0.0-10.43)	2.1 (0.37-11.11)	0.4
STa/Stx2e/AIDA	0 (0.0-10.43)	6.3 (2.19-17.16)	0.14
STa/Stx2e	0 (0.0-10.43)	6.3 (2.19-17.16)	0.14

Table 3. Adhesion factors from isolates that harboured virulence genes

Adhesion factors	ATG (n=33)	NAG (n=47)	P-value
AIDA	3.0 (0.53-15.32)	23.4 (13.6-37.22)	**0.01**
PAA	18.1(8.61-34.39)	0 (0-7.55)	**<0.005**
EAE	0 (0.0-10.43)	2.1 (0.37-11.11)	0.40
F6	0 (0.0-10.43)	4.25(1.17-14.25)	0.23

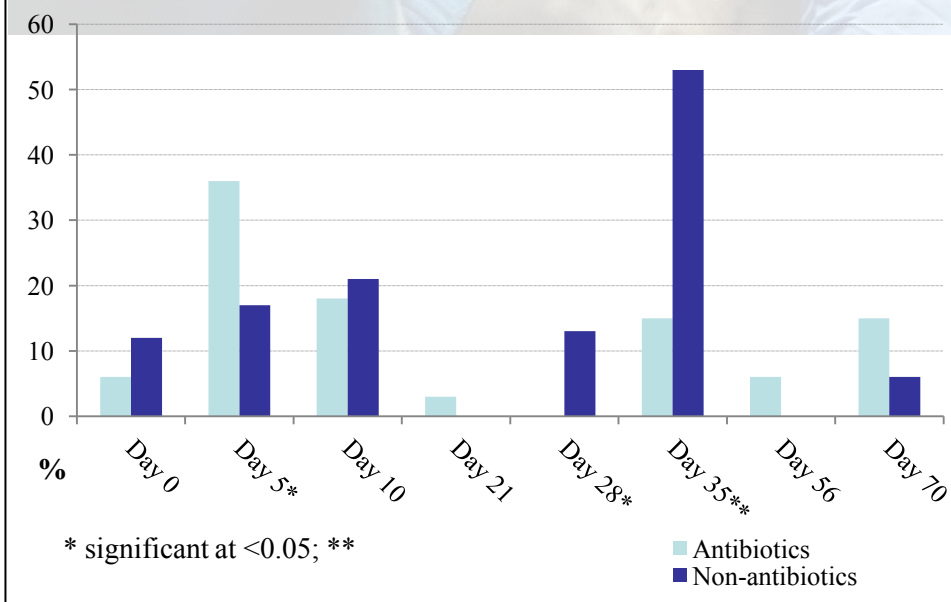

Fig 1. Frequency of isolation of virulence genes in percentages based on sampling days

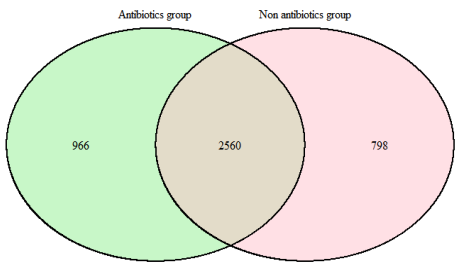

Fig 2. Venn diagram showing shared and unique OTUs in the ATG and NAG over the 70 days period.

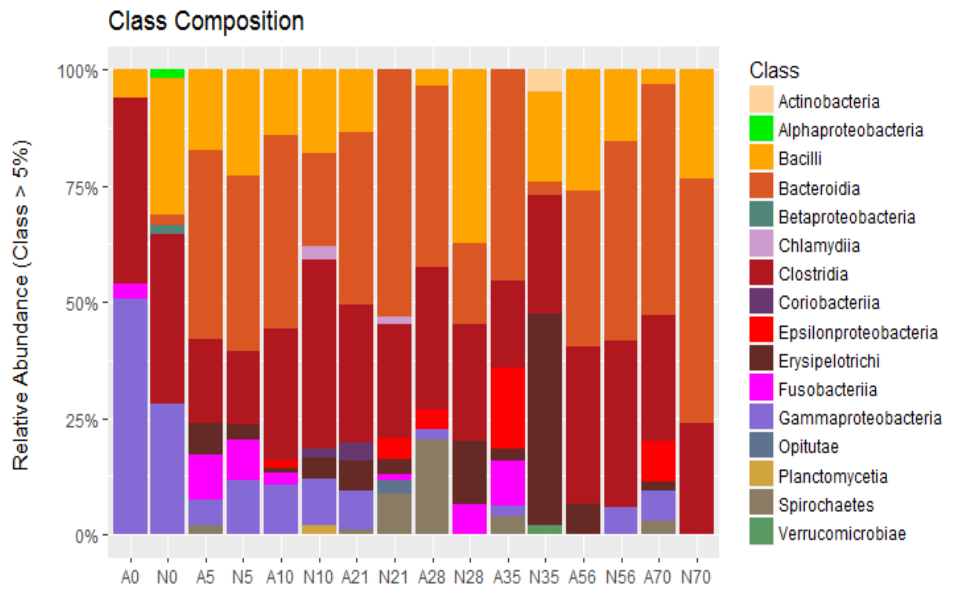

Fig 3. Relative abundance of the bacterial communities at the Class level in the ATG and NAG at all the sampling points.

Table 1. Proportion of virulence genes haboured by the *E.coli* isolates

Virulence genes	ATG (n=33)	NAG (n=47)	P-value
STa	18.1(8.61-34.39)	14.8 (7.40-27.68)	0.70
STb	0 (0.0-10.43)	8.5 (3.36-19.93)	0.09
EAST 1	78.7 (62.25-89.32)	85.1 (72.32-92.59)	0.46
StX2e	3 (0.53-15.32)	12.7 (5.98-25.17)	0.13

Conclusion:

This study showed that usage/non-usage of antibiotics in growing pigs does not prevent occurrence of disease causing virulence genes and other factors may also be involved.

References:

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