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Quantitative investigation of ESC producing E. coli in the Danish pork meat chain with estimation of the full burden of bacteria carrying blaCTX genes

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Introduction

Third and fourth generation cephalosporins are considered critical important antibiotics for treating serious infections in humans and the presence of extended-spectrum cephalosporinase (ESC)-producing bacteria in the food animal production is therefore a serious concern internationally (EFSA, 2011).

In 2010, the Danish pig industry introduced a voluntary stop for use of critically important antibiotics belonging to the group of cephalosporins. A decline in ESC resistance in pigs after the stop of using cephalosporins has been shown. By a selective enrichment procedure, Agersø and Aarestrup (2013) showed a significant reduction of the presence of ESC producing E. coli in caecal samples from pigs at slaughter, with a prevalence reduction from on average 10.9% in 2009/2010 (N=1193) to 3.5% in 2011 (N=777). The DANMAP procedure for testing random E. coli isolates from pigs and pig meat has not been designed to describe actual occurrence or differences between the years 2009-2017 (DANMAP 2009-2017).

This project aimed to provide a quantitative estimate on the prevalence and concentrations of ESC resistance carried in E. coli and in the total microbiome in the pig meat chain from slaughter to retail (Figure 1). A retail exposure assessment was defined as the quantitative occurrence of ESC resistance in 100 gram meat cuts.

Materials and Methods

During 2015 to 2018 the Danish pork chain has been investigated qualitatively and quantitatively for ESC producing E. coli and Enterobacteriaceae. The level of resistance carried by animals into slaughter was measured on caecal content (N=266). The contamination of the carcass at slaughter was measured from carcass swabs of 1400 cm². The contaminations at cutting (N= 288) and retail (N=529) were measured from meat cut samples of 100 cm². Extended-spectrum cephalosporinase (ESC)-producing E. coli and Enterobacteriaceae were culture quantified by direct plating on cefotaxime (FOT) and tetracycline (TET) containing media. A more sensitive qualitative culture analysis for ESC producing E. coli using pre-enrichment according to the standard procedure for the harmonised EU surveillance on antimicrobial resistance was also carried out.
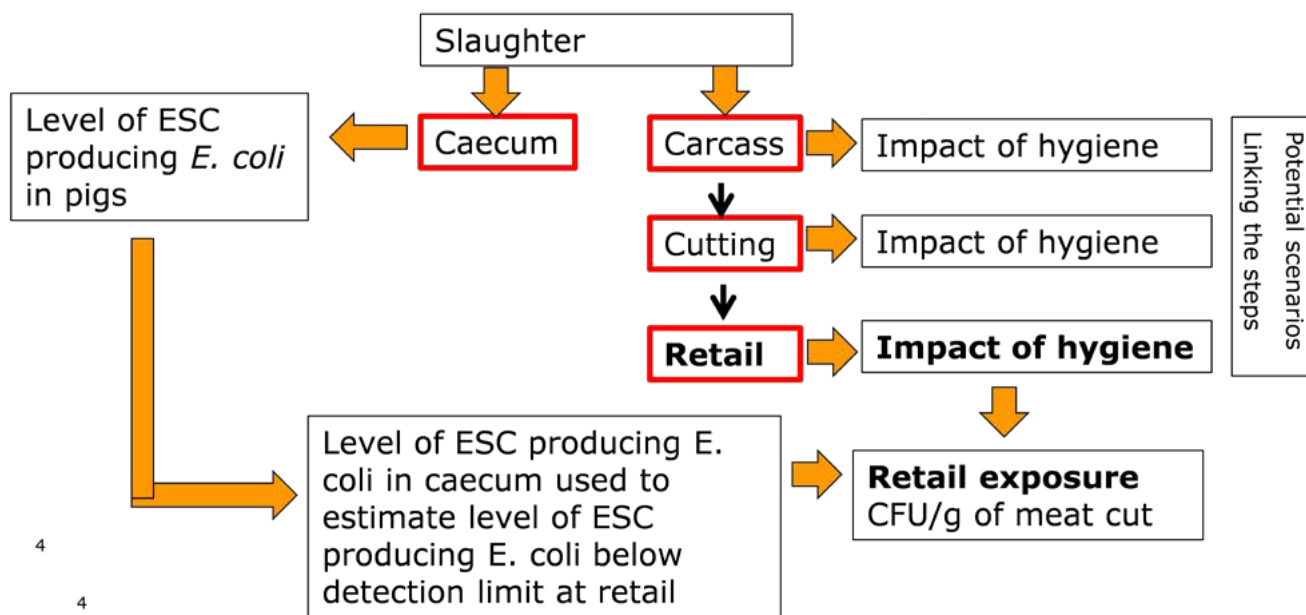

Figure 1: Diagram of elements in the ESC retail exposure assessment. Red boxes: points of data generation

To quantify the total bacterial population carrying specific resistances, qPCR was performed using primers specific for tetA , tetB, bla$_{CTX}$ genes, and for uidA (E. coli). The regression of qPCR C$_T$ values against E. coli cell counts was used to design standard curves, which enable linking of a qPCR C$_T$ value to a corresponding cell count. By this method, concentrations of bacteria carrying bla$_{CTX}$, tetA and tetB genes were estimated.

As the resistance genes analysed by qPCR target all bacteria carrying the gene, the joined data set can be used to analyse to what extent resistance occurs within E. coli compared to the total bacterial population, and how the bacterial population structure changes over the pig meat chain. The principle for quantification of the total pool of bla$_{ctx}$ genes in a sample is illustrated in Figure 2.

Maximum likelihood methods and Tobit regressions (Lorimer and Kiermeyer, 2007) were used to determine quantitative levels of ESC producing E. coli and TET resistant E. coli below the detection limit (Figure 3), which enables us to do a comparative assessment of E. coli and the total number of bacteria carrying specific ESC genes in the meat at retail. To substantiate modelling at retail, data generated at slaughter was included to support the analysis.
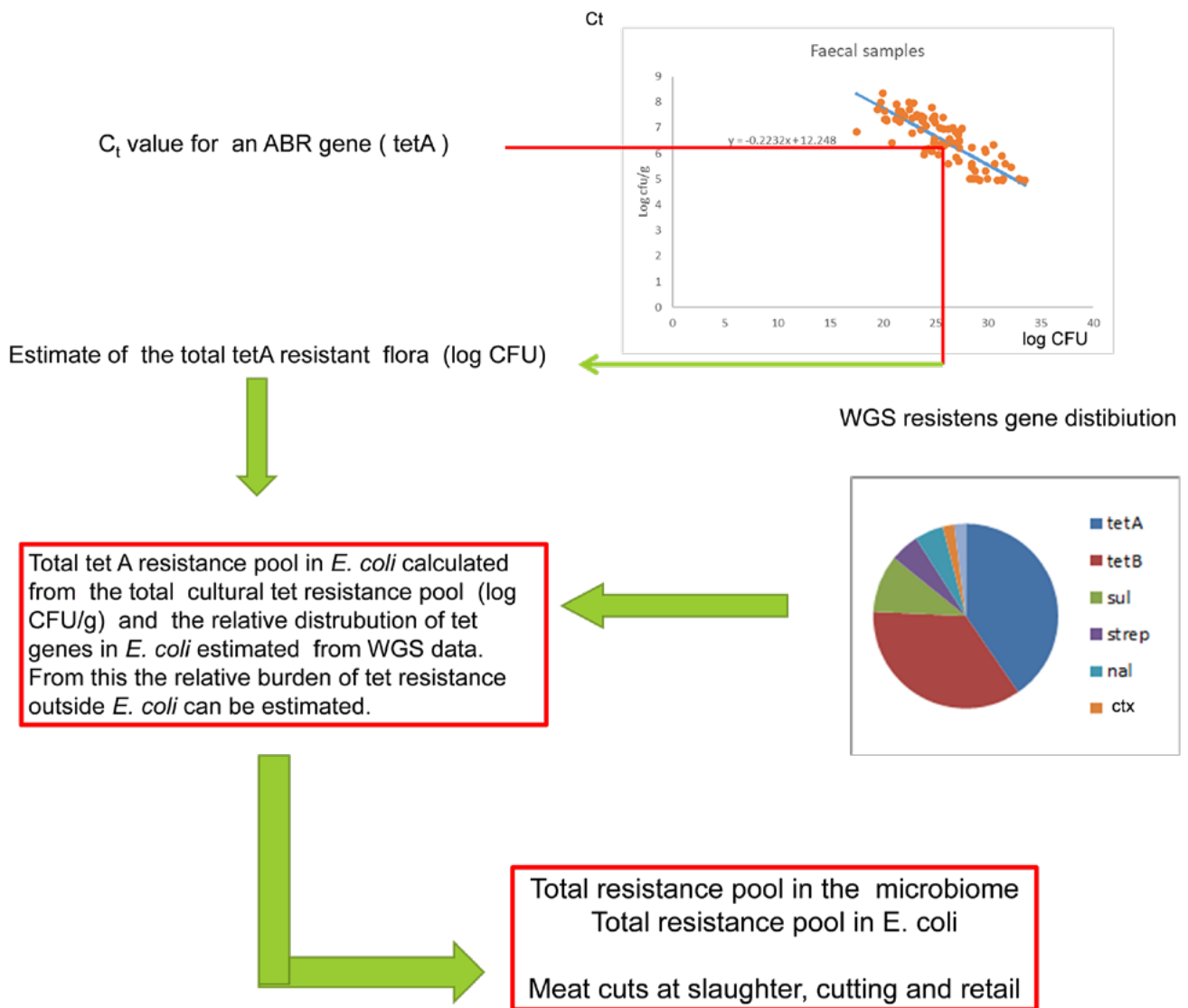

Figure 2: Principle for quantification of blactx resistance gene pool in E. coli and in the total microbiome in pig meat at slaughter, cutting and at retail
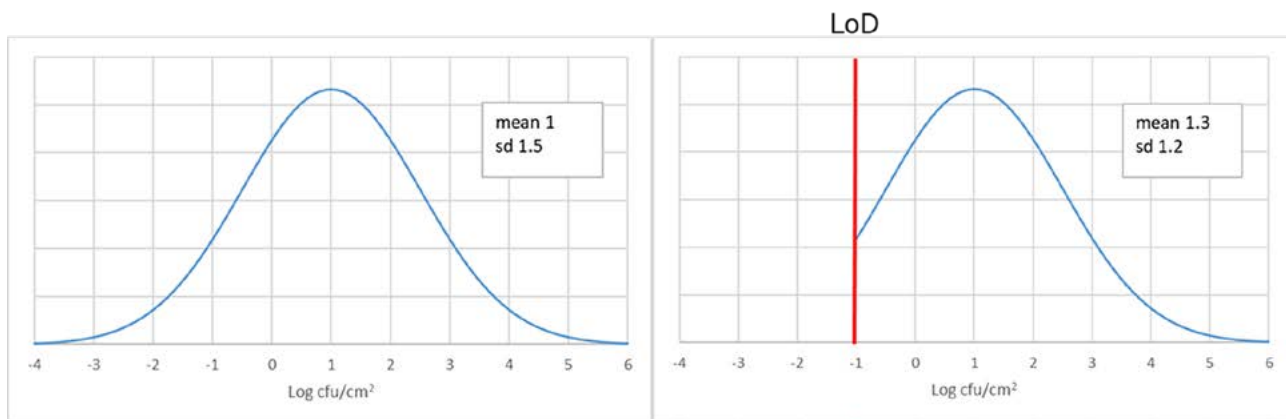
Figure 3: The (hypothetical) true distribution of concentrations (left) and the mean and standard deviation found, if the values below the LoD were not taken into account

Results

In feces, on carcasses, at cutting and at retail the observed prevalence of cefotaxime resistant E. coli was 32% , 2%, 1% and 1%, respectively. The observed mean log concentrations were 2.3 log cfu/g, 2.4 log cfu/1400 cm², -0.4 log cfu/cm², and at retail it was below the detection limit. To estimate the concentrations of ESC producing E. coli at retail we used the concentrations of E. coli and ESC producing E. coli in faecal samples for modelling mean concentration of ESC producing E. coli estimated to be -5.2 log CFU/cm2, with standard deviation 1.47. Calculating back to portions this would imply 0.2% of 100 g portions of raw pig meat at retail to be contaminated with at least 1 CFU ESC producing E. coli. The prevalence of meat being contaminated with ESC producing E.coli at 10 or 100 CFU/ 100 g was estimated to be 0.01% and 0.001%.

To compare the ESC carriage in all bacteria to that of E. coli, using Tobit regression, we estimated that the qPCR based prevalence of 100 g portions contaminated with bla_{CTX} carrying bacteria at slaughter, cutting and retail to be 4.0%, 3.6% and 0.9% respectively compared to the culture based prevalence of ESC producing E. coli of 3.6% , 3.5% and 0.2%. This indicates that a significant part of ESC resistance in pig meat is carried by E. coli.

Discussion

Despite the ban of cephalosporins for almost 10 years in the pig production, ESC resistance prevails in the Danish pork industry. The reason for this persistence is not clear, but co- and cross-resistance may play a role (Jensen et al., 2018). The use of any beta-lactam antibiotics in the primary production such as ampicillin or penicillin will select for existing ESC producing bacteria. Also, if the ESC genes do not hamper the ecological fitness of the bacteria, ESC producing bacteria may be able to sustain intestinal colonisation in pigs without any selective pressure as indicated for ESC producing E. coli in broilers (Mo et al. 2016).

Using the direct culture methods, the quantitative occurrence of ESC producing E. coli at retail was below detection limits and made it impossible to assess the retail exposure based on available culture data. This led to a novel approach, using Tobit regression, to extrapolate quantitative distributions for ESC producing bacteria below culture detection limits at retail. This extrapolation incorporated the use of data from slaughter and cutting plants to construct retail distributions. By defining a retail exposure as the level of contamination of 100 gram portions, we estimate that only 1 in 500 portions will be contaminated with at least 1 CFU ESC producing E. coli and 1 in 100.000 portions will be contaminated with more than 100 CFU. Based on qPCR amplification, we also suggest that E. coli is a major carrier of ESC genes in pig meat.

References

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