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# Prevalence of tetracycline resistance and genotypic analysis of populations of *Escherichia coli* from animals, carcasses and cuts processed at a pig slaughterhouse

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ABSTRACT

A Danish pig slaughterhouse was visited in this study to investigate the impact of carcass processing on prevalence of tetracycline-resistant *Escherichia coli*, and to identify the origins of carcass contaminations with *E. coli* by assessing genetic diversity of *E. coli* populations on carcasses. A total of 105 carcasses were sampled at five sequential stages: after stunning, after scalding, after splitting, after cooling and after cutting. Total and tetracycline-resistant *E. coli* were counted for each sample and tetracycline resistance prevalence per sample was calculated by the fraction of tetracycline-resistant *E. coli* out of total *E. coli*. From 15 repeatedly sampled carcasses, 422 *E. coli* isolates from faeces, stunned carcasses, split carcasses and chilled carcasses were examined by pulse-field gel electrophoresis (PFGE) and tested for antimicrobial susceptibility. The results showed that *E. coli* counts and the prevalence of tetracycline-resistant *E. coli* per sample were both progressively reduced after each sampling stage. PFGE analysis showed that *E. coli* populations from stunned carcasses were highly genetically diverse, compared with those from split carcasses and faeces. Thirteen carcasses (87%) were contaminated with *E. coli* that were also isolated from faeces of either the same or other pigs slaughtered on the same day; and 80% of stunned carcasses shared the same *E. coli* PFGE subtypes. The results suggest that some carcass processing steps in the slaughterhouse were effective in reducing both *E. coli* numbers and the tetracycline resistance prevalence in *E. coli* on carcasses. Faeces from the same or other pigs slaughtered on the same day were likely to be an important source of *E. coli* carcass contamination. Combined data from *E. coli* enumeration, PFGE typing and antimicrobial susceptibility testing suggest that tetracycline-susceptible *E. coli* probably persisted better compared to resistant ones during the carcass processing.



## 1. Introduction

Contamination of pork products by enteric pathogens is often a result of faecal contamination of carcasses during slaughter and processing (Warriner et al., 2002; Lindblad et al., 2007). Because of the generally small number and sporadic occurrence of pathogens, the potential sources of pathogens on carcasses are difficult to identify by enumeration. However, *Escherichia coli* are commonly found on carcasses and accepted as an indicator for possible contamination with enteric pathogens (Aslam et al., 2004; Namvar and Warriner, 2006). When trying to identify the origins of carcass contaminants, it is not possible to trace numerous strains of *E. coli* through all stages of carcass processing. Instead, the genetic diversity of *E. coli* populations on carcasses at various processing stages can be studied. For this purpose, the number of isolates recovered from each sample should be sufficient to represent most of the clones present in the sample. Changes in the genetic diversity of *E. coli* populations on carcasses can show the changes in numbers or replacement of various *E. coli* clones that result from the various operations in carcass processing and therefore indicate where the contamination with pathogens may occur.

Furthermore, the spread of antimicrobial-resistant bacteria from animals to humans via food is of great concern worldwide, as it can lead to failures of antimicrobials used for treatment of humans. Widespread use of antimicrobials with food animals is thought to make an important contribution to the development of antimicrobial resistance in bacteria (Aarestrup, 2004; Aslam and Service, 2006). Commensal bacteria, such as *E. coli*, in the intestinal tracts of food animals exposed to antimicrobial agents will inevitably develop antimicrobial resistance. Previous studies have shown that *E. coli* from slaughter animals and slaughterhouses provide a pool of antimicrobial-resistant bacteria and resistance genes (van den Bogaard et al., 2000; Aslam and Service, 2006). However, the actual impact of carcass processing on the prevalence of antimicrobial resistance has not been investigated (McMahon et al., 2007; Sidhu et al., 2002).

Random sampling of animals in a slaughter line is a common sampling strategy in slaughterhouse studies, however, few studies

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have followed individual animals through the slaughter line to track the origins of carcass contamination (Gill and Bryant, 1992; Aslam et al., 2003; Namvar and Warriner, 2006). The approach of following individual animals (repeated measurement) would present a unique opportunity to identify how carcasses are faecally contaminated and how this is associated with the introduction of specific animals. It can also provide additional data on the prevalence and persistence of particular *E. coli* clones during the carcass processing (Wonderling et al., 2003).

The objectives of this study therefore were to investigate the impact of carcass processing on the tetracycline-resistant *E. coli* on carcasses and to identify the origins of carcass contamination with *E. coli*, by assessing genetic diversity of *E. coli* populations on carcasses at various stages. Individual pigs were followed and sampled at specific stages in this study. Tetracycline-resistant *E. coli* were used as a marker for antimicrobial-resistant bacteria because of their abundant prevalence (20–40%) in Danish pigs (DANMAP, 2006).

## 2. Materials and methods

### 2.1. Sampling procedure

The slaughter line selected for this study processes about 10,000 pigs per day. Fig. 1 presents the main steps of carcass processing and sampling stages through the slaughter line. In order to ensure that any variability between herds was determined, a total of four visits (A, B, C, D) were conducted each week during November 2007. Between 25 and 30 pigs were selected per visit, resulting in 105 sampled pigs in total. Selected pigs were marked by removal of the left ear at stunning and followed throughout the processing line until the carcass was cut into three major parts. Due to restricted access to certain operation units, carcass were only sampled at five stages: after stunning, after scalding, after splitting, after chilling and after cutting. Faecal samples were collected after carcasses were eviscerated and split (Fig. 1).

All sampling was performed by the staff from the National Food Institute-Technical University of Denmark. A $10\times10$ cm piece of Cutisoft-cotton (BSN medical Ltd, Hamburg, Germany) pre-moistened with 10 ml 0.9% saline containing 0.1% peptone water (Oxoid, Basingstoke, UK) was used to swab carcass surfaces. At each sampling stage, the selected carcasses were swabbed on the same area: left hind leg of approximately $20\times20$ cm$^2$ close to the anus. About 10 g of faeces were collected directly from rectum of carcasses after splitting. Cotton swabs and faecal samples were placed into individual sterilized containers after sampling. All samples were kept cool during transportation and stored at 4 °C for 1–2 days before microbiological analysis.

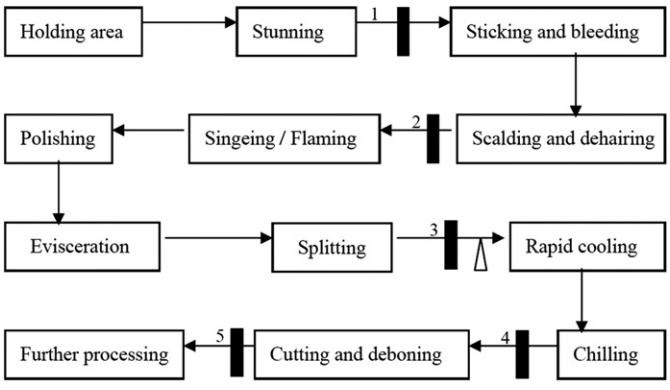


**Fig. 1.** Schematic representation of the pig slaughter line. Sampling sites for carcasses are indicated by solid bars, and the faecal sampling site is indicated by the triangle symbol.

### 2.2. Bacterial analysis

Five grams of faeces or cotton swabs were transferred to a plastic bag containing 40 ml of 0.9% sterile saline with 0.1% peptone water and homogenized for 2 min in a stomacher. Ten-fold serial dilutions were made and 100 µl of each $10^{-2}$ to $10^{-5}$ dilutions were mixed with 900 µl 0.9% saline and added to a 3M™ Petrifilm™ Select *E. coli* (SEC) plate (3M Microbiology, St. Paul., MN). In parallel, 100 µl of each $10^{0}$ to $10^{-3}$ dilutions were mixed with 900 µl 0.9% saline containing 64 mg/L oxytetracycline and added to a SEC plate. The specificity of the SEC plate medium for enumerating *E. coli* and the concentration of 64 mg/L tetracycline to discriminate tetracycline (TET)-resistant *E. coli* on the SEC plate have been validated previously (Peu et al., 2006; Mora et al., 2007; Wu et al., 2008). After incubation at 42 °C for 24 h, *E. coli* were enumerated using a Petrifilm™ Plate Reader (3M Microbiology, St. Paul., MN). Enumeration data were reported as CFU/100 cm$^2$ (carcass sample) or CFU/g (faecal sample). Tetracycline resistance in *E. coli* for each sample was measured by the within-sample prevalence of TET-resistant *E. coli*, calculated by the fraction of *E. coli* colonies on a SEC plate supplemented with oxytetracycline out of the total *E. coli* enumerated on a SEC plate without oxytetracycline for each sample (Vieira et al., 2008).

Five pigs from each of the visits B, C and D were selected for tracking *E. coli* contaminations of carcasses (pigs from Visit A were excluded to avoid possible bias because of the less proficiency in the first sampling procedure). Up to ten *E. coli* colonies were randomly picked from a SEC plate without oxytetracycline for each sample (Fig. 1). Selected colonies were sub-cultured and stored at −80 °C for pulse-field gel electrophoresis (PFGE) and antimicrobial susceptibility testing.

### 2.3. Pulse-field gel electrophoresis for the selected isolates

The selected isolates were typed by PFGE using *Xba*I enzyme (Amersham International, Amersham, UK) in accordance with the PulseNet protocol (Ribot et al., 2006).

Gels were analysed using BioNumerics V 4.5 software (Applied Maths, Sint-Martens-Latem, Belgium) and cluster analysis was performed by unweighted pair group method with averaging algorithm (UPGMA) based on the Dice similarity coefficient with a 1.5% position tolerance and a maximal optimization shift of 0.5%. Isolates with single band difference in PFGE patterns were considered as distinct and referred to as pulsotypes; isolates differing by three or fewer bands were considered highly related and assigned to the same PFGE subtype (Tenover et al., 1995); isolates were clustered using an 80% homology cut-off, above which strains were considered closely related and assigned to the same PFGE type. Genetic diversity of *E. coli* population in each sample was expressed as a diversity index (DI): number of obtained PFGE subtypes divided by number of tested *E. coli* isolates for each sample.

### 2.4. Antimicrobial susceptibility testing

Susceptibility was determined by an automated microdilution method (Trek Diagnostic Systems, East Grinstead, UK) for a panel of antimicrobial agents including tetracycline (DANMAP, 2006). The results were interpreted in accordance with CLSI guideline (CLSI, 2007).

### 2.5. Statistical analysis

*Escherichia coli* enumeration data were statistically analyzed using SAS Enterprise Guide 3.0 (SAS Institute, Cary, NC). In order to determine the effects of sampling date and the sampling stage on the total *E. coli* count and on the TET-resistant *E. coli* count, two linear regression models were developed, accounting for repeated measurements of pigs