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Campylobacter jejuni survival in a poultry processing plant environment

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ABSTRACT

Campylobacteriosis is the most common cause of bacterial gastroenteritis worldwide. Consumption of poultry, especially chicken's meat is considered the most common route for human infection. The aim of this study was to determine if *Campylobacter* spp. might persist in the poultry plant environment before and after cleaning and disinfection procedures and the distribution and their genetic relatedness. During one month from a poultry plant were analyzed a total of 494 samples -defeathering machine, evisceration machine, floor, sink, conveyor belt, shackles and broiler meat- in order to isolate *C. jejuni* and *C. coli*. Results showed that *C. jejuni and C. coli* prevalence was 94.5% and 5.5% respectively. Different typing techniques as PFGE, MLST established seven *C. jejuni* genotypes. Whole genome MLST strongly suggest that highly clonal populations of *C. jejuni* can survive in adverse environmental conditions, even after cleaning and disinfection, and persist for longer periods than previous thought (at least 21 days) in the poultry plant environment. Even so, it might act as a source of contamination independently of the contamination level of the flock entering the slaughter line.

1. Introduction

Campylobacteriosis is the most common cause of bacterial gastroenteritis in developed countries with EU-wide notification rate in 2014 of 60 cases every 100,000 persons and an estimated cost to public health systems of ~ EUR 2.4 billion a year (EFSA, 2014). Human campylobacteriosis is a self-limiting gastroenteritis that lasts for approximately 5–7 days and it is characterized by watery and sometimes bloody diarrhea, fever, abdominal cramps and vomiting (Skarp et al., 2016). Severe post-infection complications such as; reactive arthritis, bacteremia and Guillian-Barré syndrome might occur (Theoret et al., 2012).

Campylobacter jejuni and *Campylobacter coli* are responsible around 90% of all campylobacteriosis diagnosed in humans in EU (Bolton, 2015; EFSA, 2014) and they are commonly found in the gastrointestinal tract of several animal species (Epps et al., 2013). Epidemiological studies have reported chickens are the main reservoir of human campylobacteriosis. The consumption of broiler meat is considered the highest common route of human infection (Oh et al., 2015; Prachantasena et al., 2016; Young et al., 2007). *C. jejuni* and *C. coli* are fastidious microorganisms being unable to grow at temperatures below 30 °C, neither tolerating desiccation nor atmospheric levels of oxygen. They are susceptible to various environmental and food processing-induced stressors such as osmotic stress (Bronowski et al., 2014), cooking temperatures (Wanyenya et al., 2004) and different disinfectants (Gutiérrez-Martín et al., 2011). Nevertheless, both species are widely spread in the environment and can be readily isolated from contaminated food, water and other sources (Teh et al., 2014; Torralbo et al., 2014).

Once *Campylobacter* is introduced in the slaughter line, it can spread to the poultry meat, especially in the defeathering and evisceration steps (Berrang et al., 2001; Melero et al., 2012). *Campylobacter* spp. could be recovered during slaughter, in processing equipment and in the plant environment (Berndtson et al., 1996; Berrang et al., 2000; Cason et al., 2007; Ellerbroek et al., 2010). Therefore, *Campylobacter* genotypes found in the farm flock can efficiently survive and persist throughout the poultry food chain till the retail shops (Damjanova et al., 2011; Gruntar et al., 2015; Melero et al., 2012). Possible persistence of *Campylobacter*

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populations in the plant environment and their role as a continuous source of contamination is still largely unknown. Environmental persistence of *Campylobacter* in the slaughterhouse appears to be ephemeral. Poultry meat from broiler flocks otherwise negative may be contaminated if the previously slaughtered flock was positive (Allen et al., 2007; Miwa et al., 2003). However, it has been shown that negative flocks were contaminated by strains that did not generally originate from the predominating strains recovered from the ceca of the previous positive flocks (Elvers et al., 2011). Moreover, survival of *C. jejuni* overnight on food processing equipment surfaces, after cleaning and disinfection procedures, has been reported (Melero et al., 2012; Peyrat et al., 2008b). These data suggest that some strains of *Campylobacter* might survive longer in the slaughterhouse environment, and they can contaminate carcasses in several batches during the slaughter process.

Pulsed field gel electrophoresis (PFGE) has been widely used for *Campylobacter* typing (Hänninen et al., 2000; Melero et al., 2012). However, PFGE profile cannot conclusively establish the clonal relationship between isolates (Revez et al., 2014a). On the contrary, whole-genome MLST (wgMLST) has revealed as a powerful tool to resolve the relationship of especially closely related bacterial isolates by indexing allele differences in the shared loci (Kovanen et al., 2014; Revez et al., 2014a; Zhang et al., 2015), and it has been successfully applied in analyzing point-source and diffuse *Campylobacter* outbreaks (Kovanen et al., 2016; Revez et al., 2014a, 2014b).

The aims of this study were: (i) investigating the persistence of *Campylobacter jejuni* in slaughter and poultry processing environment, including food contact surfaces (FCS), non-contact surfaces (NFCS) before and after routine cleaning and disinfection procedures as well as in broiler's meat; and (ii) defining the population structure of the *Campylobacter jejuni* population isolated from different slaughterhouse and processing sampling locations and times.

2. Material and methods

2.1. Sampling procedure

A poultry slaughterhouse and processing plant in the North of Spain was studied for 4 weeks, from 2nd June to 27th June 2014. During this period, seven different farms sent the broilers to the slaughterhouse. The distance among farms varies from 4 to 125 km. Broilers were transported to slaughterhouse by crates, the previous night that they were slaughtered. Each sampling day, an average of 25.000 broilers was slaughtered from two different farms.

The slaughterhouse was visited alternately on 11 occasions during the studied period. Visits were conducted at the time of slaughter during the last part of the slaughter shift. In each visit, samples from the same sampling locations were taken before and after cleaning and disinfection. Equipment was cleaned with sodium hydroxide and potassium hydroxide and disinfected with a quaternary ammonium compound using doses and concentrations according to the supplier recommendations.

The sampling procedure was divided into two areas, taking account the ongoing activity: (1) slaughterhouse and (2) processing. Samples were classified as: food contact surfaces (FCS), non-food contact surfaces (NFCS) and broiler meat (F). During the visits, samples from the different sampling locations were collected every sampling day (Table 1). Half of those samples were taken after slaughtering, when surfaces were dirty, the another half in the following 30 min after cleaning and disinfection. As broiler meat samples, one broiler thigh was taken in the conveyor belt at the end of processing line before their distribution into the market. Carcasses of the processing area belong to flocks slaughtered the day before.

Samples from FCS and NFCS on dirty surfaces were taken using sterile sponges (3M™, Saint Paul, Minnesota, USA) except floor samples that were collected with sterile wiping towel, both were soaked with ringer solution sterile (Oxoid). Additionally, samples from clean surfaces were taken using commercial sterile premoistened sponges with neutralizing buffer (water and buffer salts) (3M™). Broiler thighs were taken entire and placed in a sterile bag. All samples were kept at 4 °C for less than 3 h before further analysis in the lab.

2.2. Isolation and identification of Campylobacter spp.

Samples were analyzed using the standard procedure (ISO 10272-1:2006), but using Preston broth instead of Bolton broth in the enrichment step (Melero et al., 2012). Each sponge and wiping towel were submerged individually in 90 mL of Preston broth made with Nutrient Broth Nº 2 (Oxoid, Basingstoke, England) supplemented with Preston *Campylobacter* Selective Supplement (Oxoid) and Campylobacter Growth Supplement Liquid (Oxoid). In the case of food samples (chicken thighs), 10 g were taken aseptically and placed in sterile stomacher bags and homogenize with 90 mL of sterile Preston broth supplemented as described above. All samples were homogenized for 120 s and incubated microaerobically using a commercial gas-generating systems-CampyGen (Oxoid) and sealed jar at 41.5 °C for 48 h. After enrichment for 48 h, a loop-full from each sample was streaked on a plate of modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) prepared with *Campylobacter* blood-free selective agar base (Oxoid) supplemented with CCDA selective supplement (Oxoid). Plates were incubated as described above for enrichment broths. From each plate, two typical isolated *Campylobacter* spp colonies were randomly selected for further analysis.

Isolated colonies from the mCCDA agar were grown on 5 mL of Brain Heart Infusion broth (Oxoid) for an overnight. DNA was extracted as previously described by Yamada et al. (2015). Briefly, strains were suspended in 100 μL of Tris-EDTA buffer (pH 8.0) and incubated at 95 °C for 10 min, and centrifuged at 16,000×*g* for 1 min. The supernatants were subsequently used as templates for PCR. All isolates were identified by Real-Time PCR following the procedure described by Hong et al. (2007) and modified by Schnider et al. (2010) to identify two target genes: *hipO* and *ceuE*. Briefly, denaturation step was conducted at 95 °C for 10 min and annealing step consisted in 40 cycles at 95 °C for 15 s and 60 °C 1 min. Primers and probes are shown in Table 2. The reactions were performed in StepOneTM (Applied Biosystems, Foster City, California, USA), and the data were analyzed by StepOneTM 2.0 Software (Applied Biosystems). As control, CECT 7572 *C. jejuni* and CECT 7571 *C. coli* were used. Confirmed *C. jejuni* and *C. coli* strains were stored for further typing at −80 °C in Brain Heart Infusion broth containing 20% of glycerol.

2.3. Pulsed field gel electrophoresis (PFGE)

C. jejuni isolates were cultured on Columbia agar (Oxoid) supplemented with 5% defibrinated sheep blood (Oxoid) below microaerobic conditions (24 h at 41.5 °C) for the purpose of PFGE genotyping. PFGE analysis, applying the restriction enzymes *Sma*I and *Kpn*I was performed according to the PulseNet International standardized protocol (www.cdc.gov/pulsenet/pathogens/pfge.html). *Kpn*I was used to check the diversity of all isolates with similar *Sma*I genotype.

Restricted DNA was electrophoresed for 22.5 h on 1% (w/v) SeaKem gold agarose in 0.5 × TBE at 6 V/cm on a Chef DR III system (Bio-Rad Laboratories, Inc., Hercules, CA, U.S.). The electrophoresis