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Higher resistance of *Campylobacter coli* compared to *Campylobacter jejuni* at chicken slaughterhouse

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ABSTRACT

In order to compare the prevalence of *Campylobacter coli* and *Campylobacter jejuni* during the processing of broilers at slaughterhouse a total of 848 samples were analyzed during 2012 in southern Spain. Four hundred and seventy six samples were collected from cloaca, carcass surfaces and quartered carcasses. Moreover, 372 environmental swabs from equipment and scalding water were collected. Minimum inhibitory concentration (MIC) to ciprofloxacin, erythromycin, streptomycin, tetracycline and gentamicin was determined for isolates from chicken meat. The general prevalence of *Campylobacter* was 68.8% (40.2% of *C. coli* and 28.5% of *C. jejuni*). The relative prevalence of *C. coli* increased from loading dock area (41.5%) to packing area (64.6%). In contrast, the relative prevalence of *C. jejuni* decreased from 58.5% to 35.4%. These differences between species from initial to final area were significant ($p = 0.02$). The highest antimicrobial resistance for *C. jejuni* and *C. coli* was detected to tetracycline (100%) and ciprofloxacin (100%), respectively. *Campylobacter coli* showed an antimicrobial resistance significantly higher than *C. jejuni* to streptomycin ($p = 0.002$) and erythromycin ($p < 0.0001$).

1. Introduction

With 220,209 human cases in the European Union, campylobacteriosis was the most commonly reported zoonosis in 2011 [1]. Undercooked poultry meat is the main source of campylobacteriosis for humans [2]. Furthermore, cross contamination from raw chicken meat through knives, cutting board or hands has been reported as a major risk factor [3].

Even though different authors have described the prevalence of *Campylobacter* in retail products [4,5], few studies include the entire production chain as far as retail [6]. *Campylobacter* can be found at all steps along the poultry production chain [7]. The evisceration process, the contact with equipment and the scalding water containing *Campylobacter* can cause multiple cross-contaminations in broiler carcasses [8,9]. In contrast, it has been reported that the number of *Campylobacter* in broiler carcasses could be reduced but not eliminated from carcasses by scalding or chilling process [10].

The prevalence of *Campylobacter* in broiler carcasses in Europe was 75.8% [11]. About two thirds of the *Campylobacter* isolates from broiler carcasses were identified as *Campylobacter jejuni*, while one third was *Campylobacter coli*. However, *C. coli* was the most frequent species isolated in Spain, Italy and Bulgaria [11].

The calculation of the minimum inhibitory concentration (MIC) by agar plate dilution method is the technique

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recommended to determine the antibiotic susceptibility for *Campylobacter* species [12]. Antimicrobial resistance has been described in *Campylobacter* isolated from dressed chickens [13] or retail meat products [7]. *Campylobacter* species have been found to be resistant to macrolides, tetracyclines and fluroquinolones [14–16], which are the main antimicrobial used to treat severe cases in human [17].

In this study, our principal aim was to determine the prevalence of *C. coli* compared to *C. jejuni* during processing at slaughterhouse. In addition, the antibiotic susceptibility for isolates from chicken meat was evaluated.

2. Materials and methods

2.1. Study design and sampling

The study was carried out along 2012 in a slaughterhouse located in southern Spain. About 60,000 chickens are slaughtered each day, with modern equipments to perform each process, as well as its own quartering room.

The slaughterhouse was divided in 6 areas to collect the samples: loading dock, scalding (water temperature: 52 °C), evisceration, classified (after air chilling for 2 h at 4 °C), quartering and final meat product/packing (room temperature: 1 °C). Swab samples were collected for 15 weeks in a row, one day each week, along the entire processing chain, from cloaca, carcass surfaces (breast and back area), quartered carcasses surfaces (breast, wing, leg and back) and slaughterhouse environment (equipment and scalding water).

A total of 848 swab samples were obtained and analyzed: 476 were taken from broilers, carcass surfaces and quartered carcasses surfaces, and 372 from equipment and scalding water (Table 1). Samples were collected in all cases from the last flock slaughtered of the sampling day by systematic random sampling in each stage. Samples were collected using sterile swabs placed in tubes containing a transport medium (Amies, Eurotubo®). Swabs were kept refrigerated until arrival at the laboratory and then processed within 24 h.

2.2. Isolation and identification of Campylobacter

Every swab was streaked onto a plate with *Campylobacter* Blood-Free Selective Agar Base (Oxoid® CM0739) added with CCDA Selective Supplement (Oxoid® SR0155).

After 48 h of incubation at 42 °C in a CO_2-enriched atmosphere achieved by AnaeroGen sachets (Oxoid®), 15–20 colonies of each plate which showed morphology compatible with *Campylobacter* were streaked onto blood agar and incubated under the same conditions as previously described.

All selected isolates were confirmed by examination of colonial morphology, Gram staining, motility in dark field microscopy, oxidase and catalase testing. Afterward, DNA extraction from bacterial cultures for isolates phenotypically classified as *Campylobacter* was performed according to the method described by Sambrook and Russell [18]. Thus, bulk colonies for each isolate in blood agar plate were taken with a loopful of 10 μl to carry out DNA extraction. Subsequently, the QIAGEN® Multiplex PCR Kit was used for the molecular identification as previously described [19].

2.3. Minimum inhibitory concentration (MIC)

For determination of the MIC, the agar dilution method was used following the protocol described by the European Committee for Antimicrobial Susceptibility Testing [20]. The cut off values used for the interpretation of the MIC results are developed by EUCAST [21]. The following antimicrobial were tested: ciprofloxacin, erythromycin, streptomycin, tetracycline and gentamicin (Sigma–Aldrich®). Eighteen double dilutions, with antimicrobial concentration obtained from 10,240 mg/L to 0.0781 mg/L, were performed.

Due to restraint budget, only a total of 60 randomly selected isolates (30 *C. coli* and 30 *C. jejuni*) from chicken meat were studied. The reference strains *C. coli* DSMZ 4689^T and *C. jejuni* ATCC 33560 were used as positive controls. Plates were incubated under microaerobic atmosphere at 42 °C. MIC values were read after 24 h of incubation.

2.4. Statistical analysis

Prevalence of *Campylobacter* species in each area as well as frequencies of susceptible isolates and comparison of susceptibility between *Campylobacter* species was calculated using SPSS v15.0 software (SPSS Inc., Chicago, IL, USA). Chi squared test was used to evaluate frequencies of *Campylobacter* species in different areas during the processing and differences in antimicrobial susceptibility between *C. coli* and *C. jejuni.*

3. Results and discussion

3.1. Changes in C. coli and C. jejuni prevalence during processing in the slaughterhouse

The general prevalence of *Campylobacter* was 68.8% (583/848), 40.2% (341/848) of *C. coli* and 28.5% (242/848) of *C. jejuni*. *Campylobacter* was isolated in all processing areas, agreeing with results reported by other authors in Spain [7] and other European countries [6].

The highest prevalence of *Campylobacter* was found in the evisceration area (92.8%) (Table 1). According to Allen et al. [9] and Frederick and Huda [22], this area is a critical control point where is required to maximize the cleaning and disinfection process. Moreover, to avoid the accidental breaking of gastrointestinal tracts and, in consequence, to decrease the contamination of carcasses, it would be necessary that the evisceration equipment is adapted to different carcass sizes [10]. However, it has been described that the most promising control strategy is to keep colonized and non-colonized flocks separate during slaughter. Furthermore, to reduce cases of campylobacteriosis in humans, meat from *Campylobacter*-positive flocks could be treated by freezing or chemical decontamination [23].

Most of the studies about the genus *Campylobacter* in broiler slaughterhouses were focussed on *C. jejuni* species