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Research article

PREVALENCE, ANTIMICROBIAL RESISTANCE, AND MOLECULAR TYPING OF THERMOPHILIC *CAMPYLOBACTER* SPP. IN A GREEK POULTRY SLAUGHTERHOUSE

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Campylobacter species are one of the leading causes of foodborne disease. Poultry is a major reservoir and source of its transmission to humans. The aim of this study was to estimate the prevalence and antimicrobial resistance of *Campylobacter spp.* isolated from chicken carcasses, the environment, and processing equipment of a poultry slaughterhouse in Greece, to identify the dominant *Campylobacter* species and to determine if there are clonal relationships among the isolates. Fifty poultry samples and 25 environmental samples were examined using microbial cultures and PCR. Forty-nine of 50 poultry samples (98%) were found to be positive for *Campylobacter* spp. The environment of the slaughterhouse was also found to be significantly contaminated with *Campylobacter* spp. Thirty-seven isolates were found to be susceptible to all antimicrobials tested (56.1%) and 29 isolates showed resistance to at least two of the antimicrobials tested (43.9%). We observed 24 different PFGE-types among the 53 isolates with 14 of them isolated only once, while five PFGE-types were represented by two isolates. The remaining 29 isolates were represented by five PFGE-types each consisting of three to 12 isolates. Regarding the relationship of the PFGE types and corresponding resistance profiles, all strains of each PFGE-type shared the same antimicrobial resistance profile. This study reports evidence for *Campylobacter* spp. cross-contamination among broiler carcasses in a Greek slaughterhouse.

Key words: *Campylobacter*, chicken, slaughterhouse, prevalence, antimicrobial resistance, molecular typing

INTRODUCTION

The presence of *Campylobacter* species on chicken carcasses is an important risk factor for human food-borne campylobacteriosis. According to the last published report of

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the European Food Safety Authority [1] campylobacteriosis is the most frequently reported zoonotic disease in humans in the European Union. The disease in humans caused by *Campylobacter* spp. is usually self – limited, mild symptoms are seen and is only lasting a few days [2]. However, in rare cases the appearance of non-gastrointestinal related complications may occur such as arthritis, neurological disorders and even the appearance of Guillain-Barré syndrome, a form of paralysis, which can lead to respiratory failure and severe neurological dysfunction, and even death [3]. Therefore, reducing the prevalence of *Campylobacter* spp. on chicken carcasses and the prevention of cross contamination with other food products is essential for the protection of public health.

Following the introduction of *Campylobacter* into a poultry farm, there is rapid transmission within the flock, which may be exacerbated by factors including stocking density and litter conditions [4,5]. Up to 100% of individual birds in a flock may be colonized by *Campylobacter*, with the highest prevalence found in the caecum. Since campylobacteriosis is not considered pathogenic in poultry and is not characterized by any symptoms, when the birds reach the desired weight they are loaded onto trucks to be transported to the processing plant [6]. Modern poultry slaughterhouses are particularly efficient and present high productivity with the ability to process up to three chicken carcasses per second. Such an intensive slaughtering process can sometimes lead to contamination of chicken carcasses with *Campylobacter* spp. due to the spillage of intestinal contents and by contact of a carcass to the other [7]. Slaughter of chickens is a serial process, which means that cross-contamination can occur at different points in the process, where birds come into contact with each other and previously contaminated equipment [7]. Chicken carcasses that carry a high number of *Campylobacter* bacteria (more than 10^3 cfu/g) pose a significant threat to public health [8] and therefore, an important intervention for the protection of consumer health would be to reduce the number of contaminated carcasses at retail level [9].

In recent years, the emergence of antibiotic resistant strains of *Campylobacter* spp., which has led to failures in the treatment of campylobacteriosis in humans, has caused great concern worldwide [10]. The widespread use of antibiotics in everyday medicine and veterinary practice has been implicated in the emergence of resistant bacteria, including *Campylobacter* spp. In livestock, especially chickens, antibiotics have been extensively used for various purposes, such as improving yields performance, and prevention and treatment of diseases [11]. This resulted in the report of a large number of animals that were raised under subtherapeutic concentrations of antibiotics [12] and caused the creation of a pool of resistant bacteria [13].

An important aspect of the problem of antimicrobial resistance is the appearance of large numbers of *Campylobacter* spp. strains resistant to fluoroquinolones and specifically to ciprofloxacin. Ciprofloxacin is one of the most popular antibiotics currently used and it is considered as the antibiotic of choice for respiratory, urological, skin, arthritic or gastrointestinal infections in adults [14]. However, in many countries such as Turkey, United Kingdom, Canada and Mexico, numerous strains of *Campylobacter* spp.

have been isolated either from humans or from chicken carcasses with a resistance rate of up to 90% to ciprofloxacin [15-18]. There is therefore an urgent need to control the prevalence of *Campylobacter* spp. in broiler carcasses and the level of antimicrobial resistance of its strains.

The aim of this study was to estimate the prevalence and antimicrobial resistance of *Campylobacter* spp. isolated from chicken carcasses and the environment of a poultry slaughterhouse in Greece. Furthermore, to identify the dominant *Campylobacter* species in the carcasses and the slaughterhouse and to determine if there are clonal relationships among the isolates in order to identify the routes of contamination and cross contamination of the carcasses.

MATERIALS AND METHODS

Sample collection

The study was conducted in a single, relatively old, but licensed and HACCP certified poultry slaughterhouse in Northern Greece, processing 6,000 birds per day. Pooled neck skins from poultry carcasses were collected in two consecutive weeks after the slaughtering process and storage in the refrigerator for two to three hours.

A sample of approximately 10 g from the neck skin was obtained from each carcass. The neck skin samples from three carcasses were pooled before the examination to form a 25 g final sample (ISO 17604:2003) [19]. A total of fifty poultry samples (coming from 150 carcasses) were examined for the presence or absence of *Campylobacter* spp. throughout the study (week 1: samples 1-25 and week 2: samples 26-50). The birds were originated from different farms for weeks 1 and 2. Also, 25 environmental samples, including five from the work surfaces right after the chill tank (samples 51-55), five from plastic containers filled with chicken carcasses (samples 56-60), five from the handles of refrigerators (samples 61-65), five from cutting boards for preparing chicken fillets (samples 66-70) and five from the palms and fingers of the employees of the slaughterhouse (samples 71-75), were taken during the working day from the slaughterhouse. About 100 cm^2 of plain surfaces, the palms and fingers of the employees and the handles of refrigerators were swabbed using a sterile cotton-tipped applicator moistened with Bolton broth. Then, the applicator was transferred to tubes containing 10 ml of Bolton broth and the same area was swabbed again with a dry applicator. Three swabs were pooled as one sample. The samples were transported to the laboratory within an hour after collection in coolers with ice and were processed immediately.

Isolation and Identification of *Campylobacter* spp.

Campylobacter spp. were isolated using the procedures detailed in ISO 10272-1:2006. Each poultry sample (25 g) was mixed with 225 ml Bolton broth in a stomacher bag, blended for 2 minutes in a Stomacher 400 laboratory blender (Seward Medical, London,

UK), and incubated under a microaerophilic environment (44 h at 42^{0}C). Swab samples were directly incubated into Bolton broth under the same conditions. A loopful (10 µl) was streaked onto Karmali agar and modified cefoperazone charcoal deoxycholate agar (mCCDA). The colonies were examined after 24 and 44 h incubation at 42^{0}C. One colony was selected from each Petri dish for further analysis and was streaked to purity on Columbia blood agar (CBA) (44 h at 42^{0}C). The isolates were then examined for morphology, motility, catalase and oxidase activity, hippurate and indoxyl acetate hydrolysis, sensitivity to nalidixic acid and cephalothin and were stored at –80^{0}C until DNA extraction and molecular identification. All media and chemicals were obtained from Merck KGaA (Darmstadt, Germany) unless otherwise stated.

Extraction of genomic DNA

Genomic DNA was isolated from bacterial cultures according to the protocol of DNA purification from Gram Negative Bacteria by the PureLink Genomic DNA kit (Thermo Fisher Scientific) used for the molecular identification of the isolates.

PCR

Campylobacter spp. were identified by PCR amplification of 16S rRNA. A fragment of 1062bp was amplified using the primer pairs as previously described [20]. *C. jejuni* were detected by the amplification of 344bp DNA fragment of hippuricase gene and *C. coli* by the amplification of 500bp DNA fragment of the aspartokinase gene using the primer pairs as previously described [20].

The PCR reactions were performed in 10µl final volume. Each reaction consisted of 1X PCR buffer (BioRon, SuperHotTaq DNA polymerase, Life Science), 3mM $MgCl_2$, 250µM of each dNTP, 300nM of each primer, 1U of SuperHot polymerase (BioRon, Life Science) and 200ng genomic DNA from the bacterial strains. The PCR started with an initial denaturation step at 94°C for 2min, followed by 30 cycles of denaturation at 94°C for 10s, annealing at 60°C for 25s and extension at 72°C for 1min and with a final extension at 72°C for 7min. The PCR amplified products were electrophorized in 1.5% agarose gels and were visualized under UV illumination by the TEX-20M after staining with ethidium bromide (Life Technologies, GibcoBRL system).

Antimicrobial susceptibility testing

Susceptibility to a panel of six antimicrobials (gentamicin, streptomycin, tetracycline, ciprofloxacin, nalidixic acid and erythromycin) was determined by a breakpoint method in Mueller-Hinton agar (Merck, Damstand, Germany) supplemented with 5% defibrinated horse blood (Merck, Damstand, Germany) as described in a previous study [21] with incubation in an microaerobic environment at 41°C for 24h. The final plate concentrations (mg/L) were: gentamicin (G) (2), streptomycin (S) (8 and 128); tetracycline (T) (2 and 128); ciprofloxacin (Cp) (1); nalidixic acid (Nx) (32); erythromycin (Er) (8). Multidrug resistance was defined as previously proposed in another study [22]. *Staphylococcus aureus* ATCC 25923 was used as the reference strain.

PFGE typing

Pulsed Field Gel Electrophoresis (PFGE) was performed using *Sma*I-digested (TaKaRa, Kyoto, Japan) fragments of bacterial chromosomal DNA according to PulseNet protocol [23]. Restriction fragments of DNA were separated by PFGE using 1% Seakem Gold agarose gels (FMC BioProducts, Rockland, MD, USA) in 0.5X Tris-borate-EDTA buffer with CHEF-DR III (Bio-Rad, Hercules, CA, USA). *Salmonella* serotype Branderup strain H9812 digested with 40 units *Xba*I (TaKaRa, Kyoto, Japan) was used as the size standard, as recommended by PulseNet. Electrophoresis conditions were 14°C for 19 h, with pulse time ranging from 6.8 to 35.4 s at an angle of 120°. Gels were stained with a solution of ethidium bromide and photographed. A database containing all the PFGE patterns were created by using Bionumerics software (version 6.6 Applied Maths, Sint-Martens-Latem, Belgium), where band patterns over the multiple gels were normalized and compared. Clustering was performed by using the Dice similarity coefficient and the unweighted pair group method with arithmetic means (UPGMA), with 1.5% of tolerance and optimization. Patterns that differed by more than one fragment were considered belonging to the distinct PFGE-type designated as CC.001, CC.002 etc.

RESULTS

Prevalence and identification of Campylobacter spp.

Forty-nine of fifty poultry pooled samples (98%) were positive for *Campylobacter* spp. The environment of the slaughterhouse was also contaminated with *Campylobacter* spp. All samples taken from the inner side of the hands of the employees of the slaughterhouse, the plastic containers, and the cutting boards were contaminated with *Campylobacter* spp. For the other two sampling points, only one sample taken from the work surfaces right after the chill tank and another one from the handles of refrigerators were contaminated. The detailed results concerning the prevalence of *Campylobacter* spp. on the carcasses and the environment of the slaughterhouse are illustrated in Table 1.

Table 1. Presence of *Campylobacter* spp. in the carcasses and the environment of a slaughterhouse, Greece, 2016

	Positive samples	Total samples	Contamination %
Carcasses	49	50	98
Work surfaces after chill tank	1	5	20
Containers with chickens	5	5	100
Refrigerator door handles	1	5	20
Cutting boards	5	5	100
Palms and fingers of employees	5	5	100

Fifty-nine isolates were identified as *Campylobacter coli*, four as *Campylobacter jejuni* and three were found to belong to another species and were characterized as *Campylobacter* spp. The analytical results are shown in Table 2.

Table 2. Molecular identification of *Campylobacter* spp.

Isolates	Primers			Species
	16s	ASP	HIP	
1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 19, 20, 21, 22, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 56, 57, 58, 59, 60, 62, 66, 69, 70, 72, 73, 74, 75	+	+	-	*C. coli* (59)
23, 67, 68,71	+	-	+	*C. jejuni* (4)
37, 38, 51	+	-	-	*Campylobacter* spp. (3)

+: PCR amplification, -: absence of PCR amplification

Antimicrobial susceptibility

The antimicrobial resistance of the sixty-six isolates (*C. coli*, *C. jejuni* and *Campylobacter* spp.) and their antimicrobial resistance profiles are illustrated in Table 3 and 4, respectively. Thirty-seven isolates were sensitive to all antimicrobials tested (56.1%) and twenty-nine were resistant to at least two of the antimicrobials (43.9%). Five distinct resistance profiles were observed among the twenty-nine resistant isolates (Table 4). Thirty-two of fifty-nine *C. coli* isolates, all four *C. jejuni* isolates (isolates 23, 67, 68, 71) and one (isolate 51) of the three unidentified *Campylobacter* isolates were found sensitive to all antimicrobials tested (Table 1). As far as the origin of the isolates is concerned, twenty-seven of the forty-nine poultry isolates were found resistant to at least two of the antimicrobials used, whereas only two (isolates 57 and 58) of the sixteen environmental isolates have showed resistance (Table 3).

Table 3. Antimicrobial resistance of the poultry and environmental *Campylobacter spp.* isolates against 6 antimicrobials

Antimicrobial resistance of the poultry and environmental *Campylobacter* spp. isolates						
Isolates	**CIPRO 1**	**ERY 8**	**GENT4**	**NAL 32**	**STR 8**	**TET 2**
1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 23, 24, 25, 26, 27, 51, 56, 59, 60, 62, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75	S	S	S	S	S	S
19, 33	R	R	R	R	R	R
20, 21, 22, 28, 29, 30, 31, 34, 46, 47, 48	R	R	S	R	R	R
32, 35, 36, 43, 44, 45, 38, 39, 40, 41	R	R	S	R	R	S
37, 42, 49, 57, 58	S	R	S	R	R	R
50	S	S	S	R	R	S
Resistant isolates	23	28	2	29	29	18
Total isolates	66	66	66	66	66	66
Resistance %	34.85	42.42	3.03	43.94	43.94	27.27

Table 4. Antimicrobial resistance profiles of the *Campylobacter* spp. isolates

Resistance profiles	*Campylobacter* spp. isolates	
	Number	%
Susceptible	37	56.06
Nx + S	1	1.51
Er + Nx + S + Te	5	7.57
Cip + Er + Nx + S	10	15.15
Cip + Er + Nx + S + Te	11	16.67
Cip + Er + Gm + Nx + S + Te	2	3.03

Nalidixic acid (Nx), Streptomycin (S), Erythromycin (Er), Tetracycline (Te), Ciprofloxacin (Cip), Gentamicin (Gm)

PFGE typing

Among fifty-nine *C. coli* isolates, fifty-three were typeable by PFGE (typeability 90%), while six isolates were excluded because their PFGE patterns were not distinguishable after multiple attempts. We also excluded from our analysis the *C. jejuni* and the *Campylobacter* spp. isolates because of their low numbers. Results from the PFGE

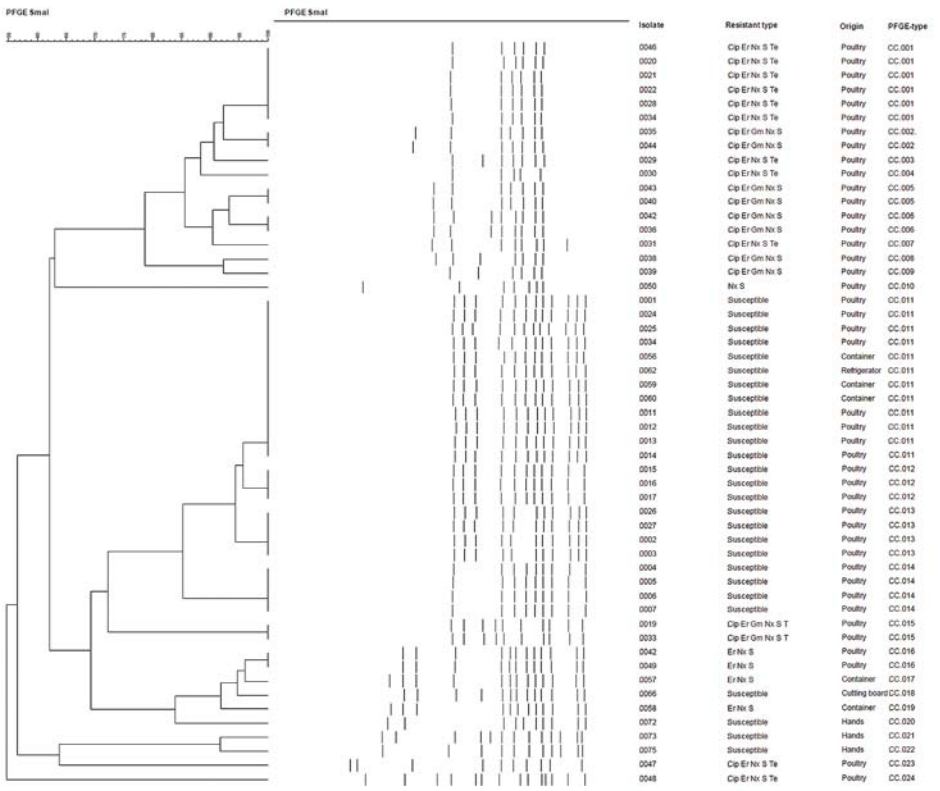

Figure 1. Dendrogram of the 24 *Sma*I PFGE types of 53 *Campylobacter coli* isolates and corresponding R-types examined in this study

analysis of the *C. coli* strains isolated from chicken carcasses and the environment of the slaughterhouse are depicted in the dendrogram of Figure 1. Overall, twenty-four different PFGE types were observed among the fifty-three isolates with fourteen of them being isolated once, while five PFGE-types were represented by two isolates. The remaining twenty-nine isolates were represented by five PFGE-types consisting of three to twelve isolates. The predominant PFGE type was CC.0011 consisting of twelve (12) isolates, followed by CC.001 consisting of six (6) isolates, CC.013 and CC.014 each one consisting of four (4) isolates and CC.012 consisting of three (3) isolates. The PFGE-type (CC.011) encompassed mostly isolates from chicken carcasses (N=8), from plastic containers (N=3) and door handles (N=1), while PFGE types CC.001, CC.012, CC.013 and CC.014 originated only from chicken isolates.

Regarding the relationship of the PFGE types and corresponding resistance profiles we observed that all the strains of each PFGE-type shared the same antimicrobial resistance profile.

DISCUSSION

This study reports the prevalence, the antimicrobial resistance and the genetic diversity of *Campylobacter* strains isolated from poultry carcasses, the environment and processing equipment of a slaughterhouse in Greece. The prevalence of *Campylobacter* on chicken carcasses was found to be very high with 98% of the carcasses being contaminated. These results, although higher, are in accordance with the recent EU summary report [1], which shows that the overall occurrence of *Campylobacter* in fresh broiler meat in the EU in 2017 was 37.4%. In the same report, a significant variation of *Campylobacter* contamination prevalence is reported, since the prevalence of *Campylobacter* contamination in chicken carcasses at the slaughterhouse level varies among Member States. Similar studies from other European countries demonstrate a variation in prevalence of contamination between different countries like Italy (5.7% and 20.7%), France (64.7%), UK (65%), Belgium (51.9%), and Estonia (35%) [24-29]. In a study reviewing seventy-three investigations of retail poultry meat all over the world, the levels of contamination by *Campylobacter* spp. were found high and extremely variable, with a total average of 58% [30]. As stated in a recent study [31], the variable prevalence of *Campylobacter* on chicken carcasses can be attributed to the type and number of samples, different methods of collection of samples, transport conditions, laboratory methods and different sanitary conditions on poultry farms and slaughterhouses.

Relating to previous investigations conducted in Greece, our findings have shown a high overall prevalence of *Campylobacter* contamination of chicken carcasses. Petridou and Zdragas suggested a prevalence of 73.3% of *Campylobacter* spp. in poultry meat in 2009 [32], while Marinou et al. (2012) reported the absence of *Campylobacter* strains in caecal samples from chicken carcasses [33]. In the most recent study conducted in Greece [34], *Campylobacter* spp. were isolated in 29.4% of the samples from free-range broilers and in 28.7% of the samples from conventional broilers.

As previously mentioned, the environment and processing equipment of the slaughterhouse that was used for this study was found to be contaminated with *Campylobacter* spp. Some of the surfaces that are regularly exposed to poultry carcasses like the plastic containers, the cutting boards and the inner side of the hands of the employees, were found contaminated and all the samples taken were positive. This finding can reflect the high prevalence of *Campylobacter* spp. on poultry carcasses and could explain the higher percentage of contamination comparing this study with previous similar studies. A flock that is contaminated with *Campylobacter* spp. can lead to the contamination of surfaces of the slaughterhouse; this also means that poultry meat from broiler flocks negative for *Campylobacter* spp. could also be contaminated if the previous slaughtered flock was positive and the bacteria remain on the surfaces of the equipment in the slaughterhouse [35]. However, *Campylobacter* spp. cannot grow and survive well in the environment for long periods [36] and is sensitive to the disinfectants that are generally used in poultry slaughterhouses [37]. This means that either there was a failure in the cleaning procedures taking place at the slaughterhouse, or that some of the flocks slaughtered within the day were contaminated and this resulted to a cross-contamination of the carcasses of the following flocks.

The distribution of the bacteria isolated to the species level revealed that the most commonly isolated *Campylobacter* species was *C. coli* (89.4%), followed by *C. jejuni* (6%) and unidentified *Campylobacter* spp. (4.6%). This observation comes in contradiction with the findings of many researchers who report that *C. jejuni* presents a higher prevalence in chicken carcasses in relation to *C. coli*. *C. jejuni* was found to be the predominant *Campylobacter* species in several studies in Greece [32, 34], France [24], Estonia [28], and Brazil [31, 38]. However, there are some other recent studies where *C. coli* and *C. jejuni* prevalence is similar [39] or even the isolation proportion of *C. coli* is higher [40]. Other studies from Greece [33], and Italy [26, 27] also report high prevalence for *C. coli*, being as high as 87.5%, 42.9% and 63%, respectively. Suzuki and Yamamoto (2009) reported that in most countries, *C. jejuni* is the dominant species isolated, although the ratio of *C. coli* to *C. jejuni* varied substantially between the countries. A recent work in Italy [41] has pointed out that the isolation proportions of *C. coli* and *C. jejuni* may vary, depending on the sampling point. In particular, they report that *C. jejuni* is significantly higher than *C. coli* on the farm, during slaughter the percentages are balanced and *C. coli* levels are higher than those of *C. jejuni* after immersion of the carcasses into the chill tank. Considering that in the present study sampling was performed after the immersion of the carcasses in the chill tank and their storage in the refrigerator, this can explain the higher proportion of *C. coli* than *C. jejuni* isolates.

Regarding the antimicrobial resistance, there was a relatively lower resistance to the six antimicrobials used in this study compared with previous studies. A recent study from Greece [34] reported that less than 20% of the *Campylobacter* isolates tested were found susceptible to all antimicrobials used in the study, while 56.1% of the isolates of this study were susceptible. Our resistance rates for ciprofloxacin (34.8%), erythromycin

(42.4%) and tetracycline (27.3%) were significantly lower than the previous study (51%, 76% and 71%, respectively) and only for gentamicin the results were comparable (3% and 0%). Nalidixic acid and streptomycin resistance rates were found to be higher than the other antibiotics used in this study, with 43.94% of the isolates being resistant to both antibiotics. These antibiotics were not tested in the previous study [34] and amikacin was used instead, representing aminoglycosides, showing a resistance rate of 15%. In a previous study from Greece [33], all *Campylobacter* isolates were found susceptible to ciprofloxacin and amoxicillin-clavulanic acid, while the resistance rates were low for nalidixic acid (14.28%) and gentamicin (14.3%) and high for erythromycin (92.8%) and ampicillin (92.8%). The *Campylobacter* isolates of this previous study originated from poultry feces at farm level, thus not providing the information of the potential *Campylobacter* isolates from carcasses after slaughter. An important finding of our study, which was confirmed by this previous study, was that *C. coli* isolates tend to be more resistant than *C. jejuni* isolates. In our study, all *C. jejuni* isolates were susceptible to the antibiotics used. Although the number of *C. jejuni* isolates in both studies (ours and Marinou et al., 2012) was relatively low and we cannot draw a certain conclusion, this finding has also been reported by other researchers in the past [42]. However, there are other researchers who supported that *C. jejuni* showed higher resistance compared to *C. coli*, which was statistically significant for ciprofloxacin, gentamycin and norfloxacin [27].

In other Mediterranean countries like France and Italy, the resistance rates for *Campylobacter* isolates were similar or higher. A study from France [24] reports that 62% of the isolates were resistant to at least one antimicrobial agent tested, with resistance to tetracycline being the most common (53.6%), followed by ciprofloxacin (32.9%) and nalidixic acid (32.0%). In the same study, 22.7% of the isolates demonstrated simultaneous resistance to ciprofloxacin, nalidixic acid and tetracycline, a percentage that is similar to the one in our study for the same antibiotics (19.7%). A study from Italy [27] also reported resistance to tetracycline being the most common (90.7%) followed by nalidixic acid (79.1%), erythromycin (72.1%), ciprofloxacin (48.5%) and gentamycin (27.9%). A quarter of the isolates (25.6%) were found resistant to ciprofloxacin and erythromycin, 13.9% were resistant to all the quinolones tested (ciprofloxacin, norfloxacin, pefloxacin and nalidixic acid) and 9.3% were resistant to all quinolones and erythromycin. Another study, from Estonia [28], stated that 63.3% of the isolates were resistant to at least one antibiotic, with the fluoroquinolone resistance rate being 41.8% and 8.2% of the isolates being resistant to ciprofloxacin, nalidixic acid and tetracycline simultaneously. Finally, in Brazil, the highest resistance rates for fluoroquinolones were reported, with 100% of the isolates being resistant to ciprofloxacin and enrofloxacin [31].

A noteworthy genomic variability among the fifty-three *C. coli* isolates was observed in this study; this was demonstrated by the large number (twenty-four) of distinct PFGE patterns indicating multiple sources of contamination. Diversity in unrelated epidemiological strains has also been observed by several authors [43,44] suggesting

that there is a diverse population of *C. coli* circulating in the poultry meat industry. We observed that for twelve isolates sharing the same PFGE type, eight of them were from chicken carcasses, three from the plastic container and one from door handles. This indicates cross contamination through the slaughter procedure. Other studies suggest that the source of contamination is mainly the chicken flock, and that cross contamination in the slaughter house is also taking place [45]. Furthermore, Sasaki et al. suggest that cross contamination can also happen between birds slaughtered on different days [46]. This was confirmed in our study since chicken and environmental isolates coming from different sampling dates were found to belong to the same PFGE type. Therefore, there was either a cross contamination between birds slaughtered on different days due to cleaning failure, or the sources of contamination of the different birds were the same, causing the infection of the birds by the same *Campylobacter* strains.

We also observed that all the isolates belonging to a certain PFGE type shared common antimicrobial resistance profiles, with no exceptions. Even isolates that were taken on different sampling days and belonged to the same PFGE type were found to have the same antimicrobial resistance profile. This strengthens our observation for clonal circulation during the slaughter procedure.

CONCLUSION

This study performs evidence of *Campylobacter* cross-contamination among broiler carcasses in a slaughterhouse. The prevalence of *Campylobacter* in chicken carcasses was observed to be very high and that was the case for some surfaces of the slaughterhouse that are regularly exposed to the chicken carcasses. An increased emphasis on cleaning combined with improved personal and general hygiene in the slaughterhouse could reduce the cross-contamination of the birds and potentially lower the percentages of contaminated poultry, improving the safety of chicken products. With respect to the antibiotic resistance observed, there was a relatively lower resistance to the antimicrobials used in this study compared with previous studies; this could actually reflect the outcomes of the efforts of the scientific community and industry over the last few years for a more prudent use of antibiotics in the food-producing animals. However, antimicrobial resistance remains a wide spread threat emphasizing the need for continuous surveillance and monitoring of the use of antimicrobial agents in husbandry.

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Authors' contributions

SI conceived the study, carried out the microbiological analysis, participated in the PCR, PFGE and antimicrobial susceptibility testing and drafted the manuscript. PT carried out the PFGE and antimicrobial susceptibility testing and helped to draft the manuscript. BE and EL carried out the PCR and helped to draft the manuscript. SG and ZA participated in the design of the study and helped to draft the manuscript. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. European Food Safety Authority EFSA, European Centre for Disease Control and Prevention ECDC: The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015. EFSA Journal 2016, 14(12):e04634-n/a.
2. Man SM: The clinical importance of emerging *Campylobacter* species. Nat Rev Gastroenterol Hepatol 2011, 8(12):669-685.
3. Hughes RA, Cornblath DR: Guillain-Barre syndrome. Lancet 2005, 366(9497):1653-1666.
4. Collett SR: Nutrition and wet litter problems in poultry. Animal Feed Science and Technology 2012, 173(1):65-75.
5. Torok VA, Hughes RJ, Ophel-Keller K, Ali M, Macalpine R: Influence of different litter materials on cecal microbiota colonization in broiler chickens. Poult Sci 2009, 88(12):2474-2481.
6. Humphrey S, Chaloner G, Kemmett K, Davidson N, Williams N, Kipar A, Humphrey T, Wigley P: *Campylobacter* jejuni Is Not Merely a Commensal in Commercial Broiler Chickens and Affects Bird Welfare. mBio 2014, 5(4).
7. Facciolà A, Riso R, Avventuroso E, Visalli G, Delia SA, Laganà P: *Campylobacter*: from microbiology to prevention. Journal of preventive medicine and hygiene 2017, 58(2):E79-E92.
8. Lindqvist R, Lindblad M: Quantitative risk assessment of thermophilic *Campylobacter* spp. and cross-contamination during handling of raw broiler chickens evaluating strategies at the producer level to reduce human campylobacteriosis in Sweden. Int J Food Microbiol 2008, 121(1):41-52.
9. Rosenquist H, Boysen L, Galliano C, Nordentoft S, Ethelberg S, Borck B: Danish strategies to control *Campylobacter* in broilers and broiler meat: facts and effects. Epidemiol Infect 2009, 137(12):1742-1750.
10. Dingle KE, Clarke L, Bowler IC: Ciprofloxacin resistance among human *Campylobacter* isolates 1991-2004: an update. J Antimicrob Chemother 2005, 56(2):435-437.
11. Landers TF, Cohen B, Wittum TE, Larson EL: A Review of Antibiotic Use in Food Animals: Perspective, Policy, and Potential. Public Health Reports 2012, 127(1):4-22.

12. Franco DA, Webb J, Taylor CE: Antibiotic and Sulfonamide Residues in Meat: Implications for Human Health. Journal of Food Protection 1990, 53(2):178-185.
13. Smith KE, Besser JM, Hedberg CW, Leano FT, Bender JB, Wicklund JH, Johnson BP, Moore KA, Osterholm MT: Quinolone-resistant *Campylobacter* jejuni infections in Minnesota, 1992-1998. Investigation Team. N Engl J Med 1999, 340(20):1525-1532.
14. Dryden MS, Gabb RJ, Wright SK: Empirical treatment of severe acute community-acquired gastroenteritis with ciprofloxacin. Clin Infect Dis 1996, 22(6):1019-1025.
15. Abay S, Kayman T, Otlu B, Hizlisoy H, Aydin F, Ertas N: Genetic diversity and antibiotic resistance profiles of *Campylobacter* jejuni isolates from poultry and humans in Turkey. Int J Food Microbiol 2014, 178:29-38.
16. Agunos A, Leger D, Avery BP, Parmley EJ, Deckert A, Carson CA, Dutil L: Ciprofloxacin-resistant *Campylobacter* spp. in retail chicken, western Canada. Emerg Infect Dis 2013, 19(7):1121-1124.
17. Strachan NJ, Forbes KJ: The growing UK epidemic of human campylobacteriosis. Lancet 2010, 376(9742):665-667.
18. Zaidi MB, McDermott PF, Campos FD, Chim R, Leon M, Vazquez G, Figueroa G, Lopez E, Contreras J, Estrada-Garcia T: Antimicrobial-resistant *Campylobacter* in the food chain in Mexico. Foodborne Pathog Dis 2012, 9(9):841-847.
19. International Organization for Standardization: Microbiology of food and animal feeding stuffs—carcass sampling for microbiological analysis ISO 17604:2003. In. Geneva: International Organization for Standardization; 2009.
20. Persson S, Olsen KE: Multiplex PCR for identification of *Campylobacter coli* and *Campylobacter jejuni* from pure cultures and directly on stool samples. J Med Microbiol 2005, 54(Pt 11):1043-1047.
21. Clinical and Laboratory Standards Institute CLSI: Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard 8th edition CLSI document M11-A8. In., edn. Wayne, PA: CLSI; 2012.
22. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B et al: Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology and Infection 2012, 18(3):268-281.
23. Ribot EM, Fitzgerald C, Kubota K, Swaminathan B, Barrett TJ: Rapid pulsed-field gel electrophoresis protocol for subtyping of *Campylobacter jejuni*. J Clin Microbiol 2001, 39(5):1889-1894.
24. Guyard-Nicodeme M, Rivoal K, Houard E, Rose V, Quesne S, Mourand G, Rouxel S, Kempf I, Guillier L, Gauchard F *et al*: Prevalence and characterization of *Campylobacter jejuni* from chicken meat sold in French retail outlets. Int J Food Microbiol 2015, 203:8-14.
25. Habib I, Berkvens D, De Zutter L, Dierick K, Van Huffel X, Speybroeck N, Geeraerd AH, Uyttendaele M: *Campylobacter* contamination in broiler carcasses and correlation with slaughterhouses operational hygiene inspection. Food Microbiol 2012, 29(1):105-112.
26. Mezher Z, Saccares S, Marcianò R, De Santis P, Rodas EMF, De Angelis V, Condoleo R: Occurrence of *Campylobacter* spp. in Poultry Meat at Retail and Processing Plants Levels in Central Italy. Italian Journal of Food Safety 2016, 5(1):5495.
27. Nobile CGA, Costantino R, Bianco A, Pileggi C, Pavia M: Prevalence and pattern of antibiotic resistance of *Campylobacter* spp. in poultry meat in Southern Italy. Food Control 2013, 32(2):715-718.

28. Meremäe K, Mäesaar M, Kramarenko T, Häkkinen L, Roasto M: The prevalence, counts and antimicrobial suspectibility of thermophilic *Campylobacter* spp. in fresh chicken meat at Estonian retail level. Agraarteadus 2015.
29. Anonymous: A UK survey of *Campylobacter* and *Salmonella* contamination of fresh chicken at retail sale. Food Survey Information sheet 04/09. In. Edited by Agency FS; 2009.
30. Suzuki H, Yamamoto S: *Campylobacter* contamination in retail poultry meats and by-products in the world: a literature survey. J Vet Med Sci 2009, 71(3):255-261.
31. Panzenhagen PHN, Aguiar WS, da Silva Frasão B, de Almeida Pereira VL, da Costa Abreu DL, dos Prazeres Rodrigues D, do Nascimento ER, de Aquino MHC: Prevalence and fluoroquinolones resistance of *Campylobacter* and *Salmonella* isolates from poultry carcasses in Rio de Janeiro, Brazil. Food Control 2016, 61:243-247.
32. Petridou E, Zdragas A: Investigation upon the presence of *Campylobacter* spp. in poultry meat and their antimicrobial resistance in Northern Greece. . In: *16th World veterinary poultry association Congress: 2009; Morocco*; 2009: p419.
33. Marinou I, Bersimis S, Ioannidis A, Nicolaou C, Mitroussia-Ziouva A, Legakis NJ, Chatzipanagiotou S: Identification and Antimicrobial Resistance of *Campylobacter* Species Isolated from Animal Sources. Frontiers in Microbiology 2012, 3:58.
34. Economou V, Zisides N, Gousia P, Petsios S, Sakkas H, Soultos N, Papadopoulou C: Prevalence and antimicrobial profile of *Campylobacter* isolates from free-range and conventional farming chicken meat during a 6-year survey. Food Control 2015, 56:161-168.
35. Allen VM, Bull SA, Corry JE, Domingue G, Jorgensen F, Frost JA, Whyte R, Gonzalez A, Elviss N, Humphrey TJ: *Campylobacter* spp. contamination of chicken carcasses during processing in relation to flock colonisation. Int J Food Microbiol 2007, 113(1):54-61.
36. Alter T, Scherer K: Stress response of *Campylobacter* spp. and its role in food processing. *J Vet Med B Infect Dis Vet Public Health* 2006, 53(8):351-357.
37. Peyrat MB, Soumet C, Maris P, Sanders P: Phenotypes and genotypes of campylobacter strains isolated after cleaning and disinfection in poultry slaughterhouses. Vet Microbiol 2008, 128(3-4):313-326.
38. Silva DT, Tejada TS, Blum-Menezes D, Dias PA, Timm CD: *Campylobacter* species isolated from poultry and humans, and their analysis using PFGE in southern Brazil. Int J Food Microbiol 2016, 217:189-194.
39. Zhu J, Yao B, Song X, Wang Y, Cui S, Xu H, Yang B, Huang J, Liu G, Yang X *et al*: Prevalence and quantification of *Campylobacter* contamination on raw chicken carcasses for retail sale in China. Food Control 2017, 75:196-202.
40. Khoshbakht R, Tabatabaei M, Hosseinzadeh S, Shirzad Aski H, Seifi S: Genetic Characterization of *Campylobacter Jejuni* and C. coli Isolated From Broilers Using flaA PCR-Restriction Fragment Length Polymorphism Method in Shiraz, Southern Iran. Jundishapur J Microbiol 2015, 8(5):e18573.
41. Marotta F, Garofolo G, Di Donato G, Aprea G, Platone I, Cianciavicchia S, Alessiani A, Di Giannatale E: Population Diversity of *Campylobacter jejuni* in Poultry and Its Dynamic of Contamination in Chicken Meat. BioMed Research International 2015, 2015:10.
42. Gallay A, Prouzet-Mauléon V, Kempf I, Lehours P, Labadi L, Camou C, Denis M, de Valk H, Desenclos J-C, Mégraud F: *Campylobacter* Antimicrobial Drug Resistance among Humans, Broiler Chickens, and Pigs, France. Emerging Infectious Diseases 2007, 13(2):259-266.
43. Vidal AB, Colles FM, Rodgers JD, McCarthy ND, Davies RH, Maiden MCJ, Clifton-Hadley FA: Genetic Diversity of *Campylobacter jejuni* and *Campylobacter* coli Isolates from

Conventional Broiler Flocks and the Impacts of Sampling Strategy and Laboratory Method. Applied and Environmental Microbiology 2016, 82(8):2347-2355.

44. Pergola S, Franciosini MP, Comitini F, Ciani M, De Luca S, Bellucci S, Menchetti L, Casagrande Proietti P: Genetic diversity and antimicrobial resistance profiles of *Campylobacter coli* and *Campylobacter jejuni* isolated from broiler chicken in farms and at time of slaughter in central Italy. J Appl Microbiol 2017, 122(5):1348-1356.
45. Normand V, Boulianne M, Quessy S: Evidence of cross-contamination by *Campylobacter* spp. of broiler carcasses using genetic characterization of isolates. Canadian Journal of Veterinary Research 2008, 72(5):396-402.
46. Sasaki Y, Maruyama N, Zou B, Haruna M, Kusukawa M, Murakami M, Asai T, Tsujiyama Y, Yamada Y: Campylobacter cross-contamination of chicken products at an abattoir. Zoonoses Public Health 2013, 60(2):134-140.

PREVALENCIJA, ANTIMIKROBNA REZISTENCIJA I MOLEKULARNA TIPIZACIJA TERMOFILNIH *Campylobacter* spp U KLANICI ŽIVINE U GRČKOJ

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Campylobacter spp. su jedan od vodećih uzroka alimentarnih infekcija. Živina predstavlja glavni rezervoar i izvor ovih mikroorganizama za ljude. Cilj ove studije je bio da se proceni prevalencija i antimikrobna rezistencija *Campylobacter* spp. izolovanih sa trupova živine, okruženja, i klanične opreme u okviru jedne klanice u Grčkoj, odredi dominantna *Campylobacter* spp. i ustanovi da li postoji klonska veza između izolata. Pedeset uzoraka živine i dodatnih 25 uzoraka iz okruženja su ispitana putem kulture mikroorganizama i pomoću PCR. Četrdeset i pet od pedeset uzoraka živine (98%) je bilo pozitivno na *Campylobacter* spp. Uočena je i značajna kontaminacija okruženja. Trideset i sedam izolata je bilo osetljivo na sve testirane antimikrobne preparate (56,1%), a 29 izolata je bilo rezistentzno na najmenje dva testirana antimikrobna preparata (43,9%). Među 53 ispitana izolata bilo je 24 različita PFGE tipa, od njih 14 je izolovano samo jedan put, dok je 5 PFGE –tipova bilo predstavljeno sa dva izolata. Preostalih 29 izolata je bilo predstavljeno putemn pet PFGE- tipova koji su se sastojali od tri do 12 izolata. Što se tiče odnosa PFGE-tipova i odgovarajućeg profila rezistencije, svi sojevi svakog PFGE-tipa su delili isti profil rezistencije. Ova studija predstavlja dokaz unakrsne *Campylobacter* spp. kontaminacije unutar jedne klanice u Grčkoj.