International Journal of Food Microbiology 338 (2021) 108984

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Cross-contamination events of *Campylobacter* spp. in domestic kitchens associated with consumer handling practices of raw poultry

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ARTICLE INFO

Keywords:
Campylobacteriosis
Chicken meat
ISO 10272
Molecular typing
Food preparation
Consumer behaviour

ABSTRACT

Contaminated poultry is the major vehicle for consumer's exposure to *Campylobacter*. This study aimed to perceive potential cross-contamination events during preparation of raw poultry that can contribute to the spread of *Campylobacter* spp. in domestic kitchen environments and to understand consumers' meanings and justifications on preparation of a poultry dish at home. A total of 18 households were visited to observe consumers preparing a recipe that included poultry. Poultry samples and swabs from the kitchen surfaces and utensils, such as kitchen cloth, hand towel, sponge, cutting boards and the sink, were collected before and after food preparation and tested for the presence of *Campylobacter* spp. Genotypic characterization of 72 *Campylobacter* spp. isolates was carried out through Pulse-Field Gel Electrophoresis (PFGE). Fourteen chicken samples were contaminated with *Campylobacter* spp. (77.8%). Twelve consumers (66.6%) washed the chicken meat under running tap water and eight (44.4%) used cutting boards. Also, only five consumers washed their hands properly prior to or during meal preparation. Cross-contamination events were detected in four kitchens, between the raw chicken and two cutting boards, two sinks and one kitchen cloth. The poultry samples presented different levels of contamination ($< 4.0 \times 10^1$ CFU/g to 2.2×10^3 CFU/g), being some poultry with lower *Campylobacter* loads the origin of three cross-contamination events during food preparation. Both *C. jejuni* and *C. coli* were recovered. Molecular typing by PFGE showed a high diversity among the isolates. There were different explanations for the practice of cleaning and rinsing chicken, but, in general, it is an habit linked to what they have learned from their families. These results highlight the potential for the dissemination of *Campylobacter* strains in the domestic environment through the preparation of chicken meat and the need to raise awareness among consumers for an appropriate handling of raw poultry in order to decrease the risk of campylobacteriosis.

1. Introduction

According to the World Health Organization (WHO), the most common cause of bacterial foodborne diarrheal disease worldwide is *Campylobacter* spp. (Kirk et al., 2015). In 2019, the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) disclosed campylobacteriosis as the most reported zoonosis in the European Union (EU) during 2018. It has been so since 2005, showing an increasing trend over the years. Information provided on the species showed that all cases were caused by thermotolerant *Campylobacter* (*C. jejuni*: 83.9%, *C. coli*: 10.3%, *C. lari* 0.1%, *C. fetus*: 0.1% and *C. upsaliensis*: 0.1%) (EFSA and ECDC, 2019).

It is well established that the main source of human infection takes place during handling or consumption of contaminated food, especially poultry meat (EFSA and ECDC, 2017; Silva et al., 2011). Generally, the bacterium colonizes the cecum and colon of birds at high levels (10^6–10^8 cells) and the chickens remain colonized until slaughter (Dhillon et al., 2006; Horrocks et al., 2009; Wilson et al., 2008). Epidemiological studies of *Campylobacter* have resulted in the implementation of hygienic and biosecurity measures on rearing and slaughtering of poultry as well as diet alterations, such as the use of additives, pre- and probiotics at the farm level with the objective of reducing human exposure (Gellynck et al., 2008; Meunier et al., 2016; van de Giessen et al., 1998). Despite all efforts, *Campylobacter* is still detected in the European retail:

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https://doi.org/10.1016/j.ijfoodmicro.2020.108984
Received 6 April 2020; Received in revised form 1 November 2020; Accepted 14 November 2020
Available online 20 November 2020

37.5% of broiler carcasses out of 7441 tested positive for *Campylobacter* in 22 European countries; 28.2% of turkey carcasses out of 1115 tested positive for *Campylobacter* spp. in nine Member States; and 23.8% of poultry samples (other than broiler and turkey) out of 302 samples tested positive for *Campylobacter* spp. in eight European countries (EFSA and ECDC, 2019).

The high prevalence of *Campylobacter* in broiler meat associated with the high consumption of this type of meat makes this product a major vehicle for consumer's exposure to this bacterium (Doorduyn et al., 2010). Thermotolerant *Campylobacter* do not grow at temperatures below 30 °C, have uniquely fastidious growth requirements and are considered fragile to several environmental stresses due to the difficulty in growing and maintaining this bacterium in laboratory culture (Bronowski et al., 2014; Fitzgerald and Nachamkin, 2007). Thus, its ability to multiply outside of an animal host and in food during their processing and storage is reduced (Fitzgerald and Nachamkin, 2007; Moore et al., 2005). Cross-contamination (e.g. use of the same knife for raw meat and other food products that are ready to eat without further killing step) and poor kitchen hygiene within the household (e.g. not washing hands or cleaning surfaces/utensils properly) play a major role in its transmission (Doorduyn et al., 2010; Facciolà et al., 2017). However, the number of cells transferred from the carcass to other surfaces/hands depends on the number of bacteria on the poultry (Verhoeff-Bakkenes et al., 2008). Another possible route of infection is the consumption of undercooked poultry meat (Suarez et al., 2019). Indeed, in 2010 EFSA estimated that handling, preparation and consumption of broiler meat accounts for 20% to 30% of human cases of campylobacteriosis (EFSA, 2010). Therefore, cross-contamination needs to be prevented. After handling poultry meat, cleaning may not be as effective as consumers expect it to be, an aspect that becomes difficult to assess given microorganisms leave no visible traces of dirt to spot. The reduction of consumers' risks to exposure is possible through prevention of cross-contamination events in home kitchens, hand washing during food preparation as well as heating food products at temperatures high enough to kill microorganisms (Cogan et al., 1999; Langsrud et al., 2020; World Health Organization, 2018). There is no single time/temperature profile for cooking poultry universally recommended by food safety authorities (Langsrud et al., 2020). According to Membré et al. (2007), the performance objective of the cooking process should be a 5.58 log reduction of *Salmonella* spp. corresponding to a core temperature of a chicken piece of 70 °C for 0.25–0.43 s.

Although cross-contamination and transfer of *Campylobacter* spp. from chicken to surfaces have been well studied and established, few studies have been performed in real scenarios, at consumers' houses, during handling and preparation of naturally contaminated poultry (Cogan et al., 1999; Guyard-Nicodème et al., 2013), or to include transdisciplinary research combining microbiological and observational analysis (Redmond et al., 2004). Therefore, the objectives of this research were to identify possible cross-contamination events that can contribute to spreading of *Campylobacter* spp. in domestic kitchen environments during real food preparation sessions, to observe consumer's handling practices of raw poultry in their own familiar environments (e.g. own kitchens at home), to understand consumers' meanings and justifications on what is safe or unsafe preparation of a poultry dish at home (e.g. how and why they do certain tasks in a particular way and not another, what are their concerns regarding poultry preparation, how do they avert certain perceived risks, and do not notice other potential risks) and to grasp what kinds of knowledge are mobilized to prepare poultry (e.g. knowledge gained from school, their parents and family relatives, friends, media, from everyday life experience).

2. Materials and methods

2.1. Household recruiting and transdisciplinary fieldwork

The observational fieldwork included one main visit to the 18 informants' households in Porto, Portugal. This visit lasted approximately 4 h to each participant household and focused on four specific stages: consumer's shopping routine (where the consumer chose and bought the food products analysed in this study according to their food preferences); transportation of food between supermarket and home; food storage routine; food preparation; and cleaning. In this study, the focus was centred on the experimental settings and results of the "food preparation" stage.

A transdisciplinary methodology to collect observational, discursive and microbial data was applied. Thus, qualitative methods used by sociologists, such as walking-with video interviews and semi-structured observation combined with Hazard Analysis and Critical Control Points (HACCP) methodology (identifying the most important steps that consumers should be aware of, here named as Consumer Critical Handling – CCH) and sampling (e.g. microbial, as explained previously) used by microbiologists were implemented (Rossvoll et al., 2012). Both social scientists and microbiologists conducted the observational work.

Observations of food preparation sessions were carried out in the participants' private kitchens, and food, surface and utensils samples were collected for microbial analysis. In the beginning of the fieldwork study (October 2017), with the aim to establish standard protocols for microbial sampling and analysis, as well as the methodological principles, tools and procedures applied, three pilot studies (Pilot A, Pilot B, and Pilot C) were conducted. For this pilot test, the three participants were recruited by snowball sampling (existing study subjects recruited future subjects from their social networks, such as family relatives, friends, neighbours or work colleagues but respecting the criteria of getting one participant from each group of risk (young man, families with children and elderly family). Subsequently, 15 additional households (P1 to P15) were recruited for the final study after discussions among partners, refinement and modification of protocols and observational templates. These visits were performed between February and April 2018 through a subcontracted professional recruitment agency. Households were selected based on recruitment criteria to include specific demographic groups identified as either vulnerable (elderly, young children and pregnant women/young families with children) or of high risk (young men - due to more frequent poor food preparation skills) (Medeiros et al., 2001). Indeed, young people are considered as high risk given that they harbor less than elderly sufficient skills in order to decrease the cross contamination likelihood in the kitchen. Participants were informed about the objectives of the study and procedures involved and were required to sign the consent form before they were formally enrolled in the study. Detailed information on pilot and experimental households is presented in Table 1. Further information in

Table 1
Detailed information on pilot and experimental households investigated in this study.

Code	Sampling date	Target group	Income	Location
Pilot A	02/10/2017	Elderly	n/a	Urban
Pilot B	03/10/2017	Single man (<30 year old)	n/a	Urban
Pilot C	03/10/2017	Family	n/a	Urban
P01	19/02/2018	Pregnant/Family	Medium	Urban
P02	20/02/2018	Pregnant/Family	Medium	Rural
P03	21/02/2018	Elderly	High	Urban
P04	26/02/2018	Elderly	Refusal	Urban
P05	27/02/2018	Family	High	Urban
P06	28/02/2018	Elderly	Refusal	Rural
P07	05/03/2018	Elderly	Medium	Urban
P08	06/03/2018	Family	Medium	Urban
P09	07/03/2018	Single man (<30 year old)	High	Urban
P10	19/03/2018	Elderly	Low	Urban
P11	20/03/2018	Family	Low	Rural
P12	21/03/2018	Single man (<30 year old)	Medium	Urban
P13	03/04/2018	Single man (<30 year old)	High	Urban
P14	05/04/2018	Elderly	Low	Urban
P15	05/04/2018	Pregnant/Family	Medium	Rural

n/a – not available.

Supplementary Table 1.

2.2. Food preparation sessions in domestic kitchens and sampling procedures

The food preparation sessions were carried out in the home kitchens of the participants who were asked to prepare a recipe with poultry and a raw vegetable salad of their own choice, the way they would normally do. The observation guide for these sessions was extensive and included questions, things or events to look for during observations, highlighted CCHs, as well as cue points on when and what to take a picture of. It also included instructions regarding kitchen and cooking and the steps to look for (e.g. cross-contamination of food, bodies and utensils).

The cooking session started by informing what the microbiologists would be doing and by explaining that sociologists would be filming the procedure, but focusing on hands and not including the face and other identifying personal traits. During the cooking phase, the social scientist was responsible for keeping the conversation going, aiming at getting the participant to talk about what they were doing while cooking. The microbiologist was also participating, but to some lesser degree. Conversation was, for the most part, allowed to flow during the cooking activity, with the focus of discussion often switching between the meal being prepared and wider topics, often unrelated to food. The social science researcher would intermittently ask for explanations about the food preparation as it unfolded (e.g. when watching someone rinsing raw chicken the sociologist would ask when time was right whether this was something habitual and why). The microbiologist took samples of chicken while the participant was cooking, always asking politely. The microbiologist also took photographs and asked some specific questions set out in the microbiology fieldwork guide complementing well the work of social scientists. The chicken samples were collected while the consumers were preparing the product for cooking, before any adding of seasoning. Participants were asked to choose raw poultry parts and to place it inside sterile plastic bags upon request. In every food preparation session, the domestic kitchen was sampled immediately before the participants had started the food preparation and after their normal cleaning procedures.

When the cooking part was over, the microbiologist would ask questions about cleaning. Some participants ate their meal immediately after cooking, while others preferred to wait until after the researchers had left. In most cases there was an opportunity to observe washing up and cleaning the kitchen, either during the cooking of the meal or afterwards. When this was not possible the research team would ask participants to simulate how would they usually clean the kitchen. The session ended with a short interview regarding pending questions that were not asked before, namely food safety and foodborne illness, which would have been inappropriate to ask during observation. Samples were taken from pre-determined sites, including: tap handle; cabinet, drawer and refrigerator handles; the counter top surface and sink. Other surfaces were sampled depending on observed behaviours during the individual food preparation sessions, for example: cutting boards (before and after food preparation if used by the participant, and after cleaning procedures but only if hand-washed). At the end of the sessions, the kitchen's cloth and/or sponge (if used), and hand towel (if touched with poorly cleaned hands after handling raw chicken) were also collected and placed into sterile plastic bags. In the three pilot households, sampling sites were swabbed with a sterile cotton tipped swabs, pre-moistened in a sterile isotonic salt solution (Ringer solution, Biokar Diagnostics, Solabia Group, Pantin, France) using aseptic techniques; the swabs were subsequently placed in sterile 13 ml plastic tubes. In experimental households, sampling sites were swabbed with sterile swab cloths (SodiBox Swab Cloths, Nevez, France). Upon completion, the cloths were carefully placed back in their original plastic bag.

Before leaving, the participant was given a voucher for their time during the one visit. Each of the above stages of observation was filmed using just one video camera, hand-held by the social science researcher. All observational and qualitative data collected was translated, processed and archived. The interviews and videos were analysed according to the techniques of social scientists (e.g. thematic and content analysis).

Food and surface swab samples were kept in coolers in the field while sampling was being completed, then immediately taken to the laboratory and stored at 4 °C until microbial testing (within 18 h).

2.3. Campylobacter spp. detection and enumeration

Campylobacter detection was performed according to International Organization for Standardization (ISO) 10272-1:2017 (International Organization for Standardization, 2017a). Briefly, food samples were aseptically weighed into sterile stomacher bags and sterile Bolton broth (VWR Chemicals, Leuven, Belgium) with 5% defibrinated horse blood (Thermo Fisher Scientific, Massachusetts, USA) was added in the proportion of x to 9× (minimum 10 g – weight used in this detection). After homogenization for 1 min in a stomacher (Interscience, Saint Nom, France), the samples were incubated for 48 h at 41.5 °C under microaerophilic conditions (10% carbon dioxide (CO_2), 5% oxygen (O_2), and 85% nitrogen (N_2)). Sampling site swabs from the pilot households were homogenized with 10 ml of sterile Bolton broth with 5% defibrinated horse blood in the stomacher for 1 min. Swabbing cloths from the experimental households and kitchen cloths were homogenized with 25 ml of sterile buffered peptone water (BPW, Bio-Rad Laboratories, California, USA) in a stomacher for 1 min, while sponges were homogenized with 50 ml of BPW, due to the foaming during homogenization, and hand towels were homogenized with 225 ml of BPW. Afterwards, a 1 ml aliquot of the homogenate was inoculated into 9 ml Bolton broth tube with 5% defibrinated horse blood and incubated at 41.5 °C under microaerophilic conditions for 48 h. The selective solid media chosen for inoculation of the enrichment culture were Modified Charcoal Cefoperazone Deoxycholate agar (mCCD agar, VWR Chemicals) and CampyFood Agar (bioMérieux, Marcy-l'Étoile, France). Both were incubated for 48 h at 41.5 °C under microaerophilic conditions. Then, up to five typical colonies of each plate were sub-cultured in Columbia agar (Merck Millipore, Massachusetts, United States) with 5% defibrinated horse blood and incubated under the same conditions for 24 h for further confirmation.

Enumeration of *Campylobacter* spp. was performed only in poultry samples according to ISO 10272-2:2017 (International Organization for Standardization, 2017b). Twenty-five grams of poultry were added to 225 ml of sterile BPW, homogenized in a stomacher for 1 min, and enumeration was performed by spread plate count of 1 ml and 0.1 ml of buffered peptone water in mCCD agar plates. Plates were incubated under microaerophilic conditions at 41.5 °C for 48 h, before typical *Campylobacter* colonies were counted. Up to five typical colonies of each plate were then sub-cultured on Columbia agar under the same conditions for 24 h for further confirmation tests. Presumptive *Campylobacter* spp. isolates obtained from detection and/or enumeration methods were sub-cultured on mCCD agar plates and confirmed by standard procedures, including observation of haemolysis after 24 h incubation, microscopy of a freshly prepared bacterial suspension and oxidase test (Shields and Cathcart, 2010).

2.4. Confirmation of presumptive Campylobacter spp. and species determination by polymerase chain reaction

Isolates where further processed for molecular confirmation using a Polymerase Chain Reaction (PCR) assay developed by Wang et al. (2002) using *Campylobacter* species-specific primers for the three major clinically relevant *Campylobacter* spp., namely *C. jejuni*, *C. coli* and *C. lari*. DNA extraction was performed with a commercial DNA extraction kit (GRS Genomic DNA Kit – Bacteria, GRiSP Research Solutions, Porto, Portugal) using the manufacture's protocol for Gram-negative bacteria. The amplification of the 23S ribosomal RNA (rRNA) from *Campylobacter* spp., the *hipO* gene (hippuricase) from *C. jejuni* and the

glyA gene (serine hydroxymethyltransferase) from *C. coli* and *C. lari* (Stab Vida, Caparica, Portugal) was carried using a 25 μl reaction mixture containing: 1× Taq Buffer with KCl (100 mM Tris-HCl, pH 8.8, and 500 mM KCl) (Thermo Fisher Scientific, Massachusetts, USA), 2 mM of $MgCl_2$ (Thermo Fisher Scientific), 200 mM of deoxynucleoside triphosphate (dNTPs) mixture (Thermo Fisher Scientific), 1.0 U of Taq DNA polymerase (Thermo Fisher Scientific), 0.2 μM of 23S rRNA primer, 1 μM of *C. coli* primer, 0.5 μM of *C. jejuni* primer and 0.5 μM of *C. lari* primer and 1 μl of DNA template. DNA amplification was carried out in a T100 thermal cycler (Bio-Rad Laboratories) with 30 cycles of amplification, after an initial denaturation step (6 min at 95 °C) performed as follows: denaturation at 95 °C for 30 s, annealing at 52 °C for 30 s, extension at 72 °C for 30 s. A final extension was performed at 72 °C for 72 min. PCR products were separated by electrophoresis on a 1.5% (w/v) agarose gel (GRS Agarose LE, GRiSP Research Solutions) with Midori Green (Nippon Genetics Europe GmbH, Dueren, Germany) in 1× Tris-acetate-Ethylenediaminetetraacetic (EDTA) buffer (TAE) (Merck Millipore) at 80 V for 45 min. DNA extracted from three reference strains from the German Collection of Microorganisms and Cell Cultures GmbH (DSMZ) was used as positive control for the PCR assays: DSMZ 4688 (*C. jejuni*), DSMZ 4689 (*C. coli*) and DSMZ 11375 (*C. lari*).

2.5. Subtyping by pulse-field gel electrophoresis (PFGE)

Campylobacter spp. isolates were cultured on Columbia blood agar under microaerophilic conditions at 41.5 °C for 24 h. Molecular subtyping of the isolates was performed according to the PulseNet protocol (Centers for Disease Control and Prevention and PulseNet, n.d.). Reference strains used as controls were DSMZ 4688 (*C. jejuni*) and DSMZ 4689 (*C. coli*). The selected restriction enzymes for all *Campylobacter* isolates were *Sma*I and *Kpn*I (Thermo Fisher Scientific). *Salmonella* Braenderup plugs restricted with *Xba*I were used as the molecular size standard. Restricted plugs were loaded into a 1% SeaKem Gold agarose gel (Lonza Group AG, Basel, Switzerland) and electrophoresed in 0.5× Tris-Borate EDTA Buffer (TBE) (GRiSP Research Solutions), at 6 V/cm and an included angle of 120° on a Chef DR III system (Bio-Rad Laboratories). *Sma*I gel's run time was 19 h while *Kpn*I gel's run lasted for 18 h. The electrophoresis conditions used were the same as mentioned on the PulseNet protocol. Gels were stained using ethidium bromide solution (MP Biomedicals, Santa Ana, California, USA) and photographed using Gel Doc XR+ System with Image Lab Software (Bio-Rad Laboratories). BioNumerics v.7.6.2 (Applied Maths, Sint-Martens-Latem, Belgium) was used for numerical analysis of the enzymes restriction patterns and Dice coefficient was used for similarity analysis (position tolerance of 1.5%). PFGE patterns were clustered using the Dice coefficient and the unweighted pair-group method using arithmetic averages (UPGMA). Classification of isolates into different *Sma*I and *Kpn*I patterns was visually validated, and a similarity threshold of ≥98% was used to define isolates belonging to the same PFGE types, that were further designated by numbers (e.g. 001).

3. Results

During the present study, a total of 18 households were visited, three pilot households in October 2017 and 15 experimental households between February and April 2018. Different chicken, surface samples, sponges, cloths and hand towels were collected throughout food preparation and microbial analyses were performed.

3.1. Occurrence of Campylobacter spp. in poultry meat

Table 2 summarizes the results obtained for the detection and enumeration of *Campylobacter* spp. In the 18 chicken samples that were analysed during this work, four were negative (Pilot B, P05, P06 and P11), whereas *Campylobacter* spp. were detected in 14 samples, at least by one of the methods applied. This represents an occurrence of 77.8%.

Table 2
Detection and enumeration of *Campylobacter* spp. results in poultry samples collected from 18 Portuguese households.

House	Shopping place	Type of meat	Detection (in 10 g)	Enumeration (CFU/g)
Pilot A	Supermarket chain A	Free range chicken (package)	Positive	2.2×10^3
Pilot B	Supermarket chain B	Chicken breast (package)	Negative	$< 1.0 \times 10^1$
Pilot C	Supermarket chain C	Chicken breast steaks (package)	Positive	Present but $<4.0 \times 10^1$
P01	Supermarket chain C	Chicken breast steak (package)	Positive	$< 1.0 \times 10^1$
P02	Supermarket chain A (butcher)	Chicken breast steaks cut into small pieces	Positive	1.6×10^2
P03	Supermarket chain A	Free range chicken thighs (package)	Negative	1.5×10^2
P04	Supermarket chain B	Chicken thighs (packaged)	Positive	$< 1.0 \times 10^1$
P05	Supermarket chain B	Chicken breast (package)	Negative	$< 1.0 \times 10^1$
P06	Supermarket chain C (butcher)	Whole chicken without skin	Negative	$< 1.0 \times 10^1$
P07	Supermarket chain C (butcher)	Whole free-range chicken	Negative	Estimated No. 9.0×10^1
P08	Supermarket chain D (butcher)	Chicken breast	Positive	Present but $<4.0 \times 10^1$
P09	Supermarket chain B	Chicken legs (package)	Negative	Present but $<4.0 \times 10^1$
P10	Street butcher shop	Whole chicken	Negative	1.4×10^2
P11	Supermarket chain A (butcher)	Whole chicken cut into pieces and without skin	Negative	$< 1.0 \times 10^1$
P12	Supermarket chain A (butcher)	Whole chicken	Negative	Present but $<4.0 \times 10^1$
P13	Supermarket chain E	Chicken thighs (package)	Negative	4.1×10^2
P14	Supermarket chain A (butcher)	Whole chicken cut into pieces	Positive	Present but $<4.0 \times 10^1$
P15	Supermarket chain C (butcher)	Whole chicken cut into pieces	Positive	Present but $<4.0 \times 10^1$

The microbial load ranged from $<1.0 \times 10^1$ to 2.2×10^3 CFU/g with only one sample presenting results above the limit established by the Commission Regulation (EU) 2017/1495 of 23 August 2017 amending Regulation (EC) No 2073/2005) for *Campylobacter* spp. (<1000 CFU/g) (European Commission, 2017).

Sixty isolates collected from detection and enumeration techniques were confirmed to belong to the *Campylobacter* genus (by a Multiplex-PCR assay), specifically 22 *C. jejuni* and 38 *C. coli*.

3.2. Occurrence of Campylobacter spp. in environmental samples

All samples collected before the food preparation were negative for *Campylobacter* spp. After food-preparation, five positive samples were detected, namely: two cutting boards, two sinks and one kitchen cloth (Table 3). Twelve consumers (66.6%) rinsed the chicken meat under running tap water, although this is not a recommended practice, and eight (44.4%) used cutting boards to prepare the chicken. *Campylobacter* was isolated from the cutting boards of pilot houses A and C, after being used to cut raw chicken that presented different levels of contamination (i.e. 2.2×10^3 CFU/g and present but $<4.0 \times 10^1$ CFU/g, respectively), meaning that even chicken meat presenting low levels of contamination may result in *Campylobacter* spp. spreading throughout the kitchen

Table 3
Results for *Campylobacter* spp. detection in cloths, hand towels, sponges and surface samples from 18 Portuguese households.

	Pilot A *	Pilot B	Pilot C *	P01 *	P02 *	P03 *	P04 *	P05	P06	P07 *	P08 *	P09 *	P10 *	P11	P12 *	P13 *	P14 *	P15 *
Proper hand washing	No	Yes	Yes	No	Yes	No	No	No	Yes	No	No	No	No	No	No	No	Yes	No
Chicken rinsing	Yes	Yes	No	Yes	No	Yes	Yes	No	Yes	Yes	No	No	Yes	Yes	Yes	No	Yes	Yes
Cutting board after use	+	−	+	ND	ND	ND	−	−	−	−	−	ND	NA	ND	NA	ND	NA	ND
Cutting board after washing with water	NA	NA	NA	NA	NA	NA	−	NA	NA	−	NA	NA	NA	NA	NA	NA	NA	NA
Tap handle	−	−	−	−	NA	NA	−	−	−	−	−	−	−	−	−	−	−	−
Handles	−	−	−	−	NA	−	−	−	−	−	−	−	−	−	−	−	−	−
Counter top	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Sink	+	−	NA	−	NA	−	−	NA	+	−	NA	NA	−	−	−	NA	−	−
Kitchen cloth	NA	NA	−	−	−	−	−	−	−	+	−	NA	−	NA	−	−	−	−
Sponge	−	NA	−	−	−	−	−	−	NA	−	−	−	NA	−	−	NA	NA	−
Hand towel	NA	NA	NA	−	NA	NA	NA	−	NA	−	NA	NA	−	NA	NA	NA	NA	NA

NA – Not Applicable; ND – Not determined as the cutting board was only used for vegetables slicing; (+) – positive; (−) – negative; * – chicken meat positive for the presence of *Campylobacter* spp. in at least one of the performed techniques.

surfaces if mishandled. Interestingly, *C. jejuni* and *C. coli* strains were isolated from both cutting boards in these houses. In pilot A, *Campylobacter* was also isolated from the sink. This can be linked to the practice of rinsing raw poultry before cooking, which was observed during the food preparation session in this household. The sink sample collected in P06 kitchen was also positive for the presence of this pathogen and, in this case, the participant also rinsed raw poultry in the sink. One kitchen cloth collected at P07 was also contaminated and associated with unsafe handling practices confirmed by observational data, namely the direct contact of the cloth with raw poultry.

Overall, two out of the three pilot households presented environmental samples that tested positive for the presence of *Campylobacter*, while only two out of 15 experimental households exhibited positive environmental samples. From the positive environmental samples, a total of 50 isolates were collected and identified as presumptive *Campylobacter* spp. by phenotypic tests, from which 12 isolates were confirmed to belong to the genus *Campylobacter* (by Multiplex-PCR assay), nine were identified as *C. jejuni* and three as *C. coli*.

3.3. PFGE typing of Campylobacter spp. and analysis of cross-contamination events

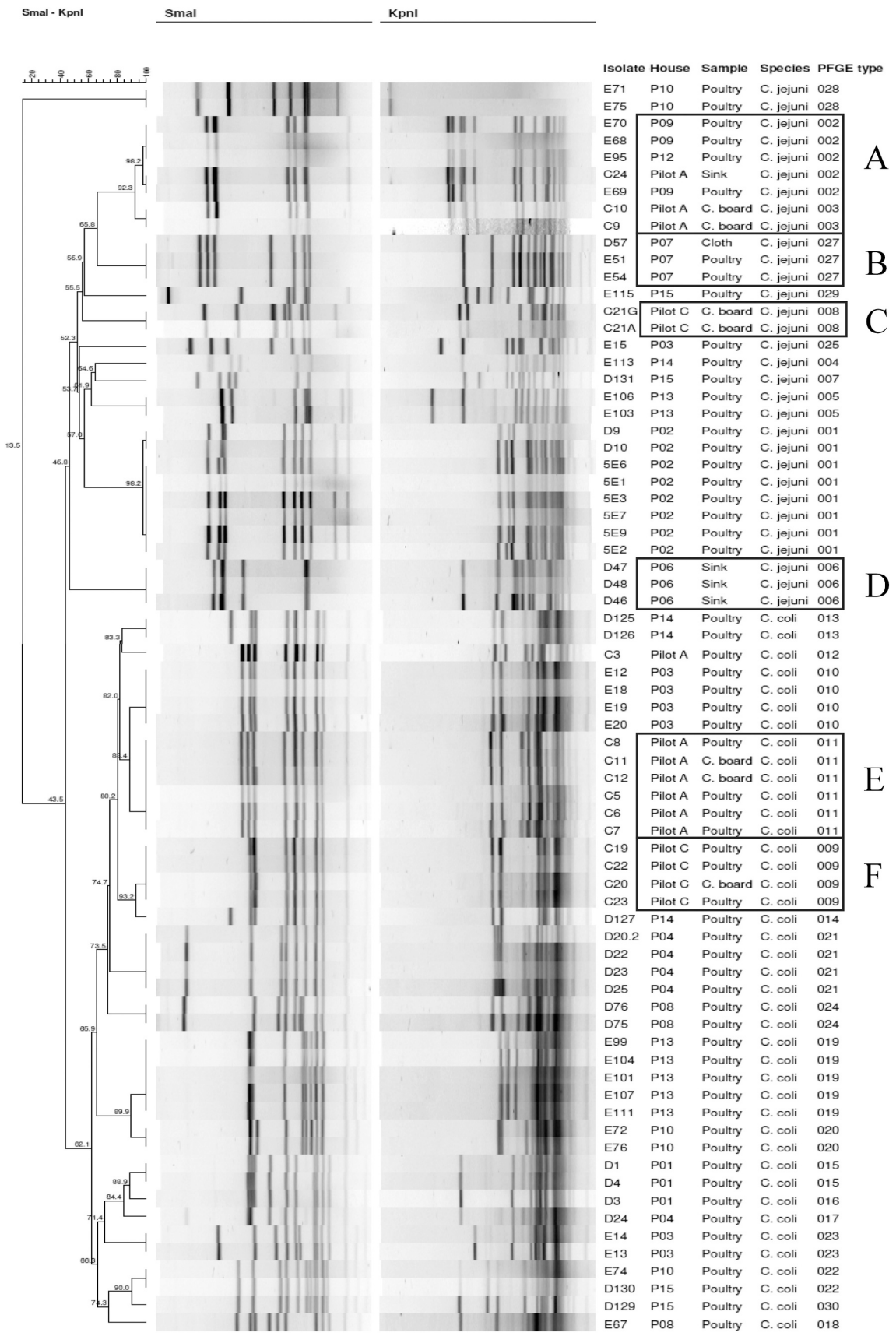
The 72 *Campylobacter* isolates (60 from detection and enumeration techniques in chicken samples and 12 from environmental samples) were characterized by PFGE typing. Restriction using *Sma*I and *Kpn*I yielded 24 and 27 different patterns, respectively, and, based on combined analysis of both enzyme's patterns, 29 PFGE types were identified, as presented in Fig. 1. However, poor additional differentiation was observed between the 72 isolates with the use of *KpnI* as a secondary enzyme, as reported by other studies (Gruntar et al., 2015; Lindmark et al., 2004). Analysis of PFGE patterns divided *C. coli* ($n = 41$) and *C. jejuni* ($n = 31$) isolates into two major independent clusters, revealing a higher genetic diversity among isolates. Seventeen unique clusters corresponded to *C. coli* and 12 to *C. jejuni*. Two *C. jejuni* isolates were untypable by *KpnI*, this phenomena has been previously reported by other authors (Gilpin et al., 2006; Oyarzabal et al., 2008).

3.4. Observational data

Our attention was paid to the conduits that enable chicken liquids and the pathogens to move about when participants were preparing chicken and salad. These conduits can be human hands, kitchen tools, cloths, surfaces and other materials used in the process.

One initial step in the course of chicken preparation is to remove the chicken from the package and place it in participants' preferred working environment (sink, kitchen surface or bowl). Participants opened the packaged chicken in various ways using fingers, knives and scissors in their efforts to open the package and move the chicken to the working environment. From all the various techniques employed, the most common was the use of a knife, followed by cooks using their hands to lift the chicken from the packaging. In these handling practices it was clear that cooks displayed and performed some level of awareness of foodborne pathogens. For example, P02 put directly the chicken in the frying pan with the minimum direct handling, using the bag that contained the chicken to avoid touching it with her bare hands; and P09 opened the pack with a knife and used it to remove the chicken from the package directly to the frying pan.

Sometimes accidents happened, thus, in order to avoid further disturbance in the kitchen, the chicken was handled in a potentially risky way. For example, P08 was preparing the chicken meal and at the same time removing the dishes from the dishwasher and storing them in kitchen cabinets. It was during multitasking that she unintendedly dropped raw chicken (that slipped off her hands) on top of a cleaned glass container she had just removed from the dishwasher. This accident



Isolate	House	Sample	Species	PFGE type	
E71	P10	Poultry	C. jejuni	028	
E75	P10	Poultry	C. jejuni	028	
E70	P09	Poultry	C. jejuni	002	
E68	P09	Poultry	C. jejuni	002	
E95	P12	Poultry	C. jejuni	002	A
C24	Pilot A	Sink	C. jejuni	002	
E69	P09	Poultry	C. jejuni	002	
C10	Pilot A	C. board	C. jejuni	003	
C9	Pilot A	C. board	C. jejuni	003	
D57	P07	Cloth	C. jejuni	027	
E51	P07	Poultry	C. jejuni	027	B
E54	P07	Poultry	C. jejuni	027	
E115	P15	Poultry	C. jejuni	029	
C21G	Pilot C	C. board	C. jejuni	008	C
C21A	Pilot C	C. board	C. jejuni	008	
E15	P03	Poultry	C. jejuni	025	
E113	P14	Poultry	C. jejuni	004	
D131	P15	Poultry	C. jejuni	007	
E106	P13	Poultry	C. jejuni	005	
E103	P13	Poultry	C. jejuni	005	
D9	P02	Poultry	C. jejuni	001	
D10	P02	Poultry	C. jejuni	001	
5E6	P02	Poultry	C. jejuni	001	
5E1	P02	Poultry	C. jejuni	001	
5E3	P02	Poultry	C. jejuni	001	
5E7	P02	Poultry	C. jejuni	001	
5E9	P02	Poultry	C. jejuni	001	
5E2	P02	Poultry	C. jejuni	001	
D47	P06	Sink	C. jejuni	006	
D48	P06	Sink	C. jejuni	006	D
D46	P06	Sink	C. jejuni	006	
D125	P14	Poultry	C. coli	013	
D126	P14	Poultry	C. coli	013	
C3	Pilot A	Poultry	C. coli	012	
E12	P03	Poultry	C. coli	010	
E18	P03	Poultry	C. coli	010	
E19	P03	Poultry	C. coli	010	
E20	P03	Poultry	C. coli	010	
C8	Pilot A	Poultry	C. coli	011	
C11	Pilot A	C. board	C. coli	011	
C12	Pilot A	C. board	C. coli	011	E
C5	Pilot A	Poultry	C. coli	011	
C6	Pilot A	Poultry	C. coli	011	
C7	Pilot A	Poultry	C. coli	011	
C19	Pilot C	Poultry	C. coli	009	
C22	Pilot C	Poultry	C. coli	009	F
C20	Pilot C	C. board	C. coli	009	
C23	Pilot C	Poultry	C. coli	009	
D127	P14	Poultry	C. coli	014	
D20.2	P04	Poultry	C. coli	021	
D22	P04	Poultry	C. coli	021	
D23	P04	Poultry	C. coli	021	
D25	P04	Poultry	C. coli	021	
D76	P08	Poultry	C. coli	024	
D75	P08	Poultry	C. coli	024	
E99	P13	Poultry	C. coli	019	
E104	P13	Poultry	C. coli	019	
E101	P13	Poultry	C. coli	019	
E107	P13	Poultry	C. coli	019	
E111	P13	Poultry	C. coli	019	
E72	P10	Poultry	C. coli	020	
E76	P10	Poultry	C. coli	020	
D1	P01	Poultry	C. coli	015	
D4	P01	Poultry	C. coli	015	
D3	P01	Poultry	C. coli	016	
D24	P04	Poultry	C. coli	017	
E14	P03	Poultry	C. coli	023	
E13	P03	Poultry	C. coli	023	
E74	P10	Poultry	C. coli	022	
D130	P15	Poultry	C. coli	022	
D129	P15	Poultry	C. coli	030	
E67	P08	Poultry	C. coli	018	

Fig. 1. Analysis of PFGE of *C. jejuni* and *C. coli* isolates characterized using *SmaI* and *KpnI* restriction enzymes. A–F: pulsotypes containing environmental isolates (similarity threshold for cluster analysis ≥98%, using UPGMA and Dice coefficient for similarity analysis with a band position tolerance of 1.5%).

and the hastiness to solve the problem of placing the raw chicken in a safe place made her carry out a series of distractive and risky food handling practices that could have possibly been averted if the accident had not happened. After touching the chicken and without washing her hands, she moved a series of small cups and other tools that were already placed on top of the chopping board and kitchen counter in order to make space to put the chicken. Distractingly, she then stored the hand-touched (and potentially contaminated) items in the kitchen cabinets without washing them again (as they were, in her mind, cleaned from the dishwasher).

The majority of consumers from this study rinsed the chicken before preparation and seasoning (12 out of 18), which reinforces the fact that rinsing chicken is not uncommon in Portuguese kitchens. Among these 12 families, all elderly participants performed this task, whereas only three young families and one single young man engaged in this activity.

During the food preparation sessions and through the conversations held with the participants we observed that participants cannot give a clear reason why they clean or rinse chicken before cooking. Some tend to say it is out of habit and it is linked to the ways their mothers handled chicken and what they have learned from their families. Thus maintain intergenerational ways of handling chicken that are passed on from mother to daughter (cooking still is a predominantly female task in Portugal). However, when they have justifications for washing the chicken these are related with the following aspects: the place where it was bought (if it is the supermarket they tend not to wash it and trust the retailers' hygiene and cleaning practices, while if it is picked up from the butcher they tend to wash it); concern that chicken meat gets spoiled very fast; the small pieces of bones that may be found in chopped chicken and the risk of chocking when eating the chicken meal; some believe that it needs rinsing because it is a 'disgusting meat' (e.g. slimy, with blood bits) or because it has 'chemicals and hormones' that need removal. In this latter case hormones and microbes that make it spoil are invisible while the sliminess can be felt in the hand through touch. Other reasons are associated with trust issues with the provisioning system (upstream practices), and also with concerns about 'properly' serving the chicken to family and friends (downstream practices). Interestingly, cleanliness, hygiene and food safety were the main reasons why people would conduct a potentially risky practice such as rinsing chicken. To illustrate with scientific evidence from the interviews the case of P06 is interesting because concerns with rinsing chicken are associated with what happens downstream when serving the meal to family, friends or guests:

Int: Do you also wash chicken breasts?

P06: I think I always wash them. Everything is washed in the house.

Int: Do you also wash steaks?

P06: No. But this I wash, because these are broken pieces and there is always bits of bones [that people can choke on]… (P06, 70 years old, elderly, rural area).

In the case of P05, it is the type of shop where the meat is bought and the packaging of the chicken that dictates whether or not down the line the chicken is rinsed.

Int: Why do you usually wash chicken from a butcher's shop?

P05: Because I have the idea that packaged chicken from the supermarket has already been sanitized whereas the chicken from the butcher has not. They cut it there. When it comes with skin, I ask to remove it. But they do not wash it there and I wash it [during the handling of the chicken meat]. If it is frozen, no. I think it gets water crystals and I always heard that freezing kills everything…" (P05, 36 years old, young family, urban area).

The ways the chicken provisioning system is organized (its food safety practices and the trust consumers place in the shop) in tandem with whether or not the chicken is packaged are factors to take into account to understand why consumers rinse raw chicken.

4. Discussion

The occurrence of *Campylobacter* spp. observed in this study was 77.8%. A lower occurrence of these microorganisms was reported for Portuguese broiler meat – 30% in 2017 (EFSA, 2018) – and in Spanish chicken products – 39.4%, of which not packed products were more contaminated than pre-packed ones (García-Sánchez et al., 2018). In order to assess the process hygiene criterion in force for food business operators in the EU, EFSA & ECDC (EFSA and ECDC, 2019) collected data concerning the microbial load of neck skin samples from chilled broiler carcasses and observed that 18.4% of 2403 tested samples exceeded the limit of 1000 CFU/g. In the present study, no difference was found between pre-packed and not packed (chosen from the supermarket's butcher shop) chicken meat but this may be due to the low number of analysed samples.

Similarly to that observed in these visits, the results from a questionnaire regarding home kitchen practices, applied to several European countries, showed that 53.7% of the 609 Portuguese respondents routinely wash their chicken in the kitchen sink (unpublished data). Chicken being rinsed seems to be a 'traditional' practice that older cooks engage in routinely, but that is not necessarily shared by younger cooks.

In addition, it was observed in the videos that participants, while cooking, are often distracted by conducting several tasks at the same time or following an automatic and non-reflective sequence of events (Warde, 2016). Not noticing that one is touching raw chicken with a cloth is part of the normal flow of everyday cooking. Performing chicken handling in a distracted and almost automatic manner is one of the ways of dealing with the complexity of multitasking when cooking. If people were compelled to think about every single step and gesture encompassed in cooking this practice would be unbearable to cope with on a daily basis.

The invisibility of microbes tricked P08 as the items did not look dirty. Moreover, the fact that those objects touched by the 'invisibly dirty hand' were being removed from a dishwasher led P08 to perceive them as clean. Interestingly, the microbiological data does not show any evidence of cross contamination by *Campylobacter* in this case. Thus, chicken handling may divert from a linear sequence of actions when they are interrupted by non-related cooking tasks, namely someone ringing the door, a phone call, a dog or a baby that needs attention, an accident that happens and diverts the course of action. All these sequences may or may not be conducive to exposure to cross contamination risks and unsafe food handling. It is this unpredictability that happens in everyday life that does not match with consumer food safety recommendations that call for more information, planning, discipline and order in the kitchen. Everyday life is composed of a flow of activities that are not linear, neither predictable nor certain, and this makes food safety recommendations to consumers a challenging task for scientists and public health professionals.

Through an inter-house perspective, it was possible to observe that five *C. jejuni* isolates collected from samples of three households (Pilot A, P09 and P12) exhibited the same PFGE pattern (i.e. PFGE type 002; Fig. 1 cluster A); in these households the chicken was bought at two different supermarket chains (A and B) but these may have the same provider. Other two *C. jejuni* isolates from Pilot A household presented 92% of similarity with this cluster (PFGE type 003). Additionally, two *C. coli* isolates from the chicken samples collected at households P10 and P15, and bought in different supermarkets, exhibited the same PFGE pattern (i.e. pulsotype 022). The remaining 27 PFGE types were unique among the isolates collected from samples at the same household. Household P02 isolates showed an overall uniform macro-restriction pattern, except for two isolates (D9 and D10). However, these presented 98.2% of similarity with the remaining isolates, so the same PFGE type was attributed to all isolates of P02. According to Tenover et al. (1995), isolates are considered to be closely related if their PFGE pattern differs by only two to three bands, being consistent with a single genetic event, i.e., a point mutation, an insertion or deletion of DNA.

Interestingly, the same chicken sample was colonized with more than one *Campylobacter* genotype, as observed by García-Sánchez et al. (2018). This happened in seven different households' chicken meat samples (Pilot A, P03, P04, P08, P10, P14 and P15), which can be explained by the rapid rate of recombination and genomic rearrangements reported within *Campylobacter* genome that hinders the establishment of a population structure and the study of long-term epidemiology (Sails et al., 2003; Wassenaar et al., 1998). Additionally, we observed that chicken samples could be colonized simultaneously with both *C. jejuni* and *C. coli*, verified in samples from Pilot A, Pilot C, P03, P10, P13, P14 and P15. These results are in accordance with other studies that report a multiple colonization in several flocks analysed (Bull et al., 2006; Hein et al., 2003; Shreeve et al., 2002).

Through an intra-house perspective and, using the analysis of the typing results combined with observational data collected during food preparation sessions, it was possible to establish cross-contamination events from the contaminated raw chicken to the kitchen environment in three households: Pilot A, Pilot C and P07. Isolates collected from cutting boards in Pilot A and Pilot C showed the same PFGE type as the isolates from the chicken samples, from the respective household (Fig. 1 clusters E and F, respectively). Additionally, in Pilot A a *C. jejuni* isolate (C24) recovered from the sink, sample, exhibited a similarity >98% with *C. jejuni* isolates (C9 and C10) recovered from the cutting board (contaminated both with *C. coli* and *C. jejuni*), although only *C. coli* isolates were isolated from the chicken sample (Fig. 1, cluster A). Hence, it is possible to infer that the suspected route of cross-contamination was the raw meat. Similarly, the cutting board of pilot C was contaminated with both *C. jejuni* (C21G and C21A – Fig. 1 cluster C) and *C. coli* (C20), while only *C. coli* isolates were isolated from the raw poultry in this household (Fig. 1 cluster F). In P07, the kitchen cloth was contaminated with the same *C. jejuni* strain found in the raw chicken.

In household P06, after the food preparation session, only the sink was found to be contaminated with *C. jejuni*. The three isolates recovered (D47, D48 and D49) presented the same genotype (Fig. 1 cluster D). As the sample collected before food preparation tested negative for the presence of *Campylobacter* spp. and the raw chicken was washed in the sink during preparation, it is believed that raw chicken was the probable source of contamination. *Campylobacter* was not detected in the meat sample, either because it was not present in the specific chicken part analysed or due to a high level of contamination by other species, that render difficult the isolation of characteristics colonies of *Campylobacter*. The hypothesis of the presence of this isolate as a result of a previous contamination episode is very unlikely as the survival and multiplication of this pathogen in the extra-intestinal environment, when exposed to air and light, has been reported to be highly impaired (Cogan et al., 1999; Fernandez et al., 1985). Through comparison of isolation methods, Oyarzabal et al. (2013) concluded that the reference method (ISO 10272) does not capture high variability of strains in a chicken sample, when compared to typing of isolates from other isolation techniques (rinsing of samples in BPW and filtration of the enrichment broth).

Similarly to the present study, other authors reported events of cross-contamination when preparing naturally contaminated chicken meat in the home kitchen environment. In a study in Ireland with 12 consumers, cross contamination was reported in 50% of these kitchens, being hands, counter top, oven handle and the draining board positive for the presence of *Campylobacter* (Gorman et al., 2002). In 52 domestic kitchens in the UK, Mattick et al. (2003) reported the survival of this microorganism in two out of 52 sponges/dishcloths/scourers and in one out of 32 hand towels during washing-up after preparation of poultry meat with 96% of *Campylobacter* occurrence. Bremer et al. (2005) conducted a survey in private households in Germany inquiring consumers on hygiene in relation to handling various types of raw meat. Respondents reported not cleaning their cutting boards with soap (48.1%) or washing their hands (46.6%) after preparing raw meat.

Additionally, it was observed in the visits' videos that only five of the 18 consumers washed their hands properly, using soap, after handling the raw poultry meat (Table 3). Recently, in a survey conducted across ten European countries, in the scope of the SafeConsume project (SafeConsume, 2017), a large number of respondents indicated that they typically touch the chicken with their bare hands when preparing it: Hungary (60.8%); Romania (55.7%); Portugal (53.7%); Greece (51.9%); Germany (46.2%); Denmark (36.9%); Norway (30.8%); Spain (27.9%); United Kingdom (25.8%); and, France (18.3%; unpublished data). Luber et al. (2006) quantified the transfer rate of *Campylobacter* spp. during poultry handling. Average cross-contamination rate from hands and kitchen utensils to ready-to-eat food ranged from 2.9% to 27.5%. However, lower percentages were noticed in transfer rates from chicken legs and fillets to hands (2.9% and 3.8%), from poultry fillets to the cutting board and knife (1.1%) and from chicken legs to the plate (0.3%). These results highlight the potential for cross contamination and survival of this foodborne pathogen in the kitchen environment and the need to raise public awareness on appropriate handling of raw chicken meat products in order to avoid foodborne illnesses.

No specific habit or factors (such as high microbial load in the meat or using less contaminated parts of the chicken carcass) could be attributed to the certainty of a cross-contamination event, since samples classified as negative generated positive environmental samples and vice-versa. However, this study shows that when consumers adopt long scientifically known risk factor (such as rinsing the chicken) this contributes to the spreading of *Campylobacter* in ordinary domestic kitchens and opens a window of opportunity towards infection.

5. Conclusion

These results highlight the potential for cross-contamination and survival of this foodborne pathogen in the kitchen environment and the need to raise public awareness on appropriate handling of raw chicken meat products. Washing the poultry before cooking may contaminate the surroundings, so it is recommended to consumers to transfer the poultry from the packaging directly to the oven/pot, without washing the meat, and to wash their hands after touching products containing raw poultry meat. Cleaning surfaces/utensils properly after use or changing utensils (e.g. knife and cutting board) should be done when preparing chicken meat. Additionally, during cleaning procedures, the consumer should use disposable cloths/paper or change cloth after use.

For such information campaigns to be more successful an understanding of consumers' practices in their kitchens is fundamental, being attentive to family dynamics; variation of kitchen practices according to material, economic, social and cultural factors; increasing consumers' capacity to access to such information in a clear and sensical way, that fits their everyday life routines. Information needs to be targeted, tailored and transformed in order to fit the dynamics of consumers' social practices.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2020.108984.

Declaration of competing interest

None.

Acknowledgements

Special thanks to the SafeConsume participants Trond Møretrø (Nofima, Norway; being in charge of the sampling for all countries), Silje Elisabeth Skuland (OsloMet, Norway) and Solveig Langsrud (Nofima, Norway; being in charge of the transdisciplinary interview guide/HACCP) and all researchers in Work Packages 1 and 2 for their scientific inputs (http://safeconsume.eu/about/timeline).

This research was supported by the Horizon 2020 project SafeConsume (Grant Agreement No. 727580). We would also like to thank the scientific collaboration under the Fundação para a Ciência e a

Tecnologia (FCT) project UID/Multi/50016/2019.

Ethics approval and consent for publication

The study had the ethical clearance from the National Commission of Data Protection in Portugal (CNPD). Ethical procedures were applied regarding confidentiality, anonymity, data protection, data use and consent for publication, data archiving and participant informed consent. Before conducting fieldwork with human participants an informed consent form was signed by all, explaining in detail the objectives of the research and indicating the contact of researchers in case participants wished to opt out from the study at a later stage.

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