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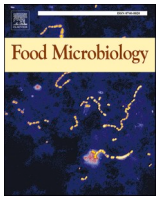

Occurrence of *Campylobacter, Listeria monocytogenes*, and extended-spectrum beta-lactamase *Escherichia coli* in slaughterhouses before and after cleaning and disinfection

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ABSTRACT

To prevent foodborne illness, adequate cleaning and disinfection (C&D) is essential to remove pathogenic bacteria from the slaughter environment. The aim of this study was to determine the presence of *Campylobacter* spp., *Listeria monocytogenes,* and extended-spectrum beta-lactamase-producing *Escherichia coli* (ESBL *E. coli*) before and after C&D in slaughterhouses.

Samples from food- and non-food contact surfaces taken before and after C&D in one red meat and one poultry slaughterhouse were analyzed for the target bacteria. Whole-genome sequencing and antimicrobial susceptibility testing were performed.

In total, 484 samples were analyzed. *Campylobacter* spp. were isolated from 13.0% to 15.5% of samples before C&D in the red meat and poultry slaughterhouse, respectively. *Listeria monocytogenes* was isolated before C&D in 12.5% and 5.2% of samples in the red meat and poultry slaughterhouse, respectively. It was noted that *C. jejuni* was detected on multiple surfaces and that *L. monocytogenes* showed potential persistence in one slaughterhouse. After C&D, *L. monocytogenes* was found in one sample. ESBL *E. coli* was not detected either before or after C&D.

These findings show the possibility to remove pathogenic bacteria from slaughter and meat processing facilities, but also indicate that deficiencies in slaughter hygiene pose a risk of cross-contamination of meat.

1. Introduction

The slaughter environment is continuously contaminated with tissues, fecal matter, blood, and other body fluids from animals during the slaughter process. Proper cleaning and disinfection (C&D) after slaughter is therefore crucial to prevent cross-contamination and spread of pathogenic bacteria. Failures in the C&D procedure can result in foodborne outbreaks, e.g., a large outbreak with five-fold higher annual reported cases of campylobacteriosis in Sweden in 2016–2017 was found to be caused by inadequate cleaning of chicken transport crates (Lofstedt, 2019). In the European Union (EU), campylobacteriosis has been the most commonly reported bacterial zoonosis in humans since 2005, in many cases associated with consumption of broiler meat (EFSA-ECDC, 2022). To control *Campylobacter* levels on broiler carcasses, a process hygiene criterion was introduced into EU legislation in 2018. It requires poultry slaughterhouses to make improvements in slaughter hygiene and to review process controls if a microbiological threshold of >3.0 log CFU *Campylobacter* per gram neck skin is exceeded (EC, 2005). To ensure compliance with this criterion, poultry slaughterhouses may also decide to sample surfaces, especially since *Campylobacter* has been shown to resist C&D procedures and persist on food contact surfaces (FCS) (i.e., defeathering machines, shackles, conveyor belts) and non-food contact surfaces (NFCS) (i.e., sinks, floors) for days to weeks, and cross-contaminate carcasses on the next slaughter day (García-Sánchez et al., 2017; Peyrat et al., 2008).

Food businesses manufacturing ready-to-eat (RTE) foods in which *Listeria monocytogenes* may pose a risk must sample equipment and the processing environment, and conduct analyses for this bacterium (EG, 2005). Food businesses producing raw meat, such as slaughterhouses,

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Abbreviations

C&D	Cleaning and disinfection
ST	Sequence type

may also decide to sample the environment for analysis of *L. monocytogenes*, since listeriosis has a high fatality rate among vulnerable consumers (EFSA Panel on Biological Hazards, 2018). *Listeria monocytogenes* is known to resist C&D procedures due to its biofilm-producing abilities (Møretrø et al., 2012; Stoller et al., 2019). In meat processing plants, *L. monocytogenes* has been shown to persist in the environment despite C&D procedures (Demaître et al., 2021; Gómez et al., 2015; Martín et al., 2014; Muhterem-Uyar et al., 2015). It is also well known that *L. monocytogenes* can cross-contaminate food and cause outbreaks due to inadequately cleaned and disinfected surfaces (Martín et al., 2014; Okpo et al., 2015; Stephan et al., 2015). There is an increasing trend in the number of confirmed human cases of listeriosis in Sweden (Public Health Agency of Sweden, 2018).

Another important food-borne bacterium that has been detected on pig, sheep, bovine, and chicken carcasses in several European countries is extended-spectrum beta-lactamase-producing *Escherichia coli* (ESBL *E. coli*) (Bardoň et al., 2013; Biasino et al., 2018; Pacholewicz et al., 2015; Tsitsos et al., 2022). One study found that ESBL *E. coli* was more abundant in the environment of a sheep slaughterhouse than on the actual carcasses (Atlaw et al., 2022). Other studies observed cross-contamination with *E. coli* caused by inadequately cleaned carcass-breaking equipment in a beef processing plant (Gill and McGinnis, 2000) and cross-contamination with ESBL *E. coli* during scalding and defeathering in a poultry slaughterhouse (Projahn et al., 2019). Other ESBL-producing bacteria have been detected in the intestines of humans and animals and constitute a global public health challenge due to the risk of increased spread of antimicrobial resistance (AMR) leading to more difficult-to-treat infections (Palmeira and Ferreira, 2020; Ninios et al., 2014; Ramatla et al., 2023).

To ensure compliance with the microbiological criteria set for food pathogens (i.e., *L. monocytogenes* and *Campylobacter* spp.) and hygiene indicator bacteria (i.e., total aerobic bacteria, *Enterobacterales*, *E. coli*) on carcasses, samples should also be taken from processing areas and equipment, by using the ISO standard 18593 as a reference. The ISO standard 18593:2018 describes surface sampling methods for both FCS and NFCS (i.e., trolleys, slicers, cutting boards, conveyor belts, drains and floors) (EG, 2005; Swedish Standards Institute, 2018). Sampling must be risk-based, meaning that sampling plans differ greatly between slaughterhouses. However, few previous investigations have evaluated the efficacy of C&D procedures in controlling the occurrence of different pathogenic bacteria in slaughterhouse environment.

The aim of this study was to determine the presence of *Campylobacter* spp., *L. monocytogenes,* and ESBL *E. coli* on surfaces before and after C&D in slaughter areas and adjacent meat processing facilities in two Swedish slaughterhouses (red meat and poultry) and to study the AMR and genetic similarity of the detected bacterial isolates. Another aim was to compare strains isolated from the present study with those detected in other studies.

2. Materials and methods

2.1. Study design

The two Swedish slaughterhouses included in the study were a small/medium-scale red meat slaughterhouse, slaughtering approximately 100–120 swine and 25 cattle per day, and a large-scale poultry slaughterhouse, slaughtering approximately 220,000 broilers per day, both with adjacent areas processing raw meat (including cutting, meat preparation, and packaging facilities). Both slaughterhouses used a rotation of alkaline chemicals (i.e., sodium hypochlorite, sodium hydroxide and potassium hydroxide) and acidic chemicals (i.e. phosphoric acid and acetic acid) for C&D, and a low-pressure water pump (approximate pressure 28–35 bar) for application of these. Each slaughterhouse was visited on six occasions, with sampling of slaughter and raw meat processing areas performed before and after C&D on each occasion (Table 1). No sampling was carried out during colder months (December–March), as *Campylobacter* prevalence can be expected to be low during this period (Hansson et al., 2007). Both FCS and NFCS, including scald water, were sampled (Table 2). This investigation formed part of a larger study reported in a previous publication, which provides detailed descriptions of selection of sampling points (Moazzami et al., 2023).

2.2. Sampling procedure

Swabbing for *Campylobacter* spp. was performed using three sterile wiping cloths pre-moistened with 30 mL Cary-Blair transport medium (National Veterinary Institute, Uppsala, Sweden). After sampling, an additional 30 mL Cary-Blair medium were added to each sample before transportation to the laboratory. Commercial pre-hydrated sponges (Hydra-Sponge 1.5 × 3 inches Sponge w/10 mL Letheen broth, 3M Health Care, St. Paul, USA) were used for swabbing for *L. monocytogenes* and ESBL *E. coli*. For practical reasons, cutting blades (sampling points 6, 12, 19), salt injector needles (sampling point 20) and "Other" sampling points (saw, display, cutting board) were sampled using commercial pre-hydrated swabs (Swab-sampler with 10 mL D/E Neutralizing broth, 3M Health Care, St. Paul, USA). Sampling was performed aseptically by using new gloves for each sampling point, avoiding contact with the sampler and immediately placing it in the commercial bag/container after sampling. On flat surfaces, when possible, sterilized stainless steel frames, stored in sterilized bags until just before use, were employed to delineate the exact sampling area. Swabbing was performed using firm and even pressure, with overlapping horizontal and vertical strokes. Approximately 45 mL of scald water were collected in a sterile plastic bottle from the upper part of the scald water tank, before C&D (directly after slaughter finished) and after C&D (immediately before the next slaughter shift started) and analyzed for *L. monocytogenes*, ESBL *E. coli,* and *Campylobacter* spp. The same individuals performed all samplings during the study. After each sampling, the samples were transported in an insulated box with refrigerant gel packs to the Animal Biosciences laboratory at the

Swedish University of Agricultural Sciences, Uppsala, Sweden. The temperature was checked upon arrival. Only samples with temperature 2–8 °C were accepted for analysis, which began within 12 h after sampling.

Table 1
Time of sampling at the two Swedish slaughterhouses investigated in this study and number of samples taken before and after cleaning and disinfection (C&D) on each sampling occasion.

Occasion	Slaughterhouse	Month	Year	No. of samples	
				Before C&D	After C&D
1	Red meat	Oct	2020	20	20
2	Poultry	Oct	2020	20	20
3	Poultry	Nov	2020	20	20
4	Red meat	Nov	2020	22	22
5	Poultry	Apr	2021	18	18
6	Red meat	May	2021	22	22
7	Poultry	May	2021	20	20
8	Red meat	May	2021	22	22
9	Poultry	June	2021	20	20
10	Red meat	June	2021	20	20
11	Red meat	Aug	2021	20	20
12	Poultry	Oct	2021	18	18
Total				242	242

Table 2
Sampling points, surface material, and sampling area/volume of food contact surfaces *(FCS)* and non-food contact surfaces *(NFCS)* in the two slaughterhouses.

Slaughterhouse	Area	Sampling point		Material	Area/volume
Red meat	**Slaughter**	**1**	Post-dehairing table pigs, upper part *(FCS)*	Stainless steel	100 cm^2
		2	Scald water pigs *(FCS)*	Liquid	1 mL
		3	Table for cattle organs, evisceration, upper part *(NFCS)*	Stainless steel	100 cm^2
		4	Conveyor belt pig organs, evisceration, upper part *(NFCS)*	Soft plastic	100 cm^2
		5	Drain cattle, carcass inspection, inside and outside *(NFCS)*	Stainless steel	100 cm^2
		6	Cutting blade cattle/pig, carcass splitting *(FCS)*	Stainless steel	20 cm^2
		Other	a	a	100 cm^2
	Processing	**7**	Cutting board beef cuts, upper part *(FCS)*	Hard plastic	100 cm^2
		8	Conveyor belt beef cuts, upper part *(FCS)*	Soft plastic	100 cm^2
		9	Conveyor belt packaging beef cuts, upper part *(FCS)*	Hard plastic	100 cm^2
		10	Trolley beef cuts, bottom *(FCS)*	Stainless steel	100 cm^2
		11	Drain below cutting board, inside and outside *(NFCS)*	Stainless steel	100 cm^2
Poultry	**Slaughter**	**12**	Cutting blade bleeding *(FCS)*	Stainless steel	100 cm^2
		13	Scald water *(FCS)*	Liquid	1 mL
		14	Plucking fingers *(FCS)*	Rubber	5 fingers (100 cm^2)
		15	Shackle after stunning *(FCS)*	Stainless steel	1 shackle (25 cm^2)
		16	Floor lairage *(NFCS)*	Concrete	100 cm^2
		Other	b	b	100 cm^2
	Processing	**17**	Conveyor belt chicken cuts, upper part *(FCS)*	Soft plastic	100 cm^2
		18	Conveyor belt chicken cuts, upper part *(FCS)*	Hard plastic	100 cm^2
		19	Cutting blade thighs *(FCS)*	Stainless steel	25 cm^2
		20	Salt injector needles *(FCS)*	Stainless steel	5 needles (25 cm^2)
		21	Drain below cutting blade thighs, inside and outside *(NFCS)*	Stainless steel	100 cm^2

[a] Saw, outside part *(NFCS)* (stainless steel); door between slaughter area and chill room *(FCS)* (hard plastic).
[b] Conveyor belt, upper part *(FCS)* (hard plastic); conveyor belt, upper part *(FCS)* (soft plastic); table for edible organs, upper part *(FCS)* (stainless steel); computer display *(NFCS)* (hard plastic); cutting board, upper part *(FCS)* (hard plastic).

2.3. Detection of Campylobacter spp.

The soaked wiping cloth samples (section 2.2) were used for detection of *Campylobacter* spp., which was performed according to ISO 10272-2 (2017) with slight modifications. Upon arrival at the laboratory, 90 mL Bolton Broth (Oxoid CM0983, Oxoid, Basingstoke, UK) with supplement (Oxoid SR0183E) and 5% Horse Blood Lysed (Håtunalab, Bro, Sweden) were added to the wiping cloths, and blended in manually from outside the bags. A 10 mL subsample of scald water was added to 90 mL Bolton broth. The samples were pre-incubated at 37 ± 1 °C for 4 h in microaerobic atmosphere using CampyGen™ (Oxoid PO5091A, Oxoid) in anaerobic jars, and then the jars were incubated at 41.5 ± 1 °C for 44 ± 4 h. After enrichment, approximately 20 μL from each sample were streaked on a plate of modified charcoal cephoperazone desoxycholate agar (mCCDA) (Oxoid CM0739, Oxoid) and incubated at 41.5 ± 1 °C for 48 ± 4 h (Fig. 1). Characteristic *Campylobacter* colonies were re-cultured on blood agar plates (National Veterinary Institute, Uppsala, Sweden) and incubated at 41.5 ± 1 °C in microaerobic atmosphere for 48 ± 4 h, and were then identified to species level using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF) (Bruker Daltonics, Billerica, Massachusetts, USA).

2.4. Analyses of Listeria monocytogenes and ESBL E. coli

Sponges were homogenized for 120 s at 240 rpm (easyMIX Lab Blender, AES-Chemunex, Weber Scientific, Hamilton, New Jersey, USA). Each swab sample and samples of scald water were vortexed for approximately 10 s. Analysis of L. *monocytogenes* was performed according to ISO 11290-1 (2018), with some modifications. For quantitative analysis, 0.1 mL of original suspension, was surface-plated on a Chromogenic *Listeria* Agar (ISO) plate (PO5183A, Thermo Fischer Scientific, Wesel, Germany) (Fig. 1). Colonies characteristic of *L. monocytogenes* were enumerated. The detection limit was 1.0 log CFU/area and for scald water 1.0 log CFU/mL. For qualitative analysis, 20 mL buffered peptone water (BPW) (Oxoid CM0509, Oxoid) were added to the original sponges and they were homogenized for 60 s at 240 rpm. From this dilution, 5–10 mL were added together with half the sponge (aseptically cut) to a stomacher bag containing 90 mL room-temperature Half Fraser broth (Oxoid CM0895B, Oxoid) with supplement (Oxoid SR0166E, Oxoid). From the original swab sampler and scald water suspensions, 5–10 mL were added to 90 mL Half Fraser broth. After incubation at 30 ± 1 °C for 24 ± 3 h, approximately a 40 μL portion was surface-plated on a Chromogenic *Listeria* Agar plate, which was incubated at 37 ± 1 °C for 24 ± 3 h (Fig. 1). Characteristic colonies were re-

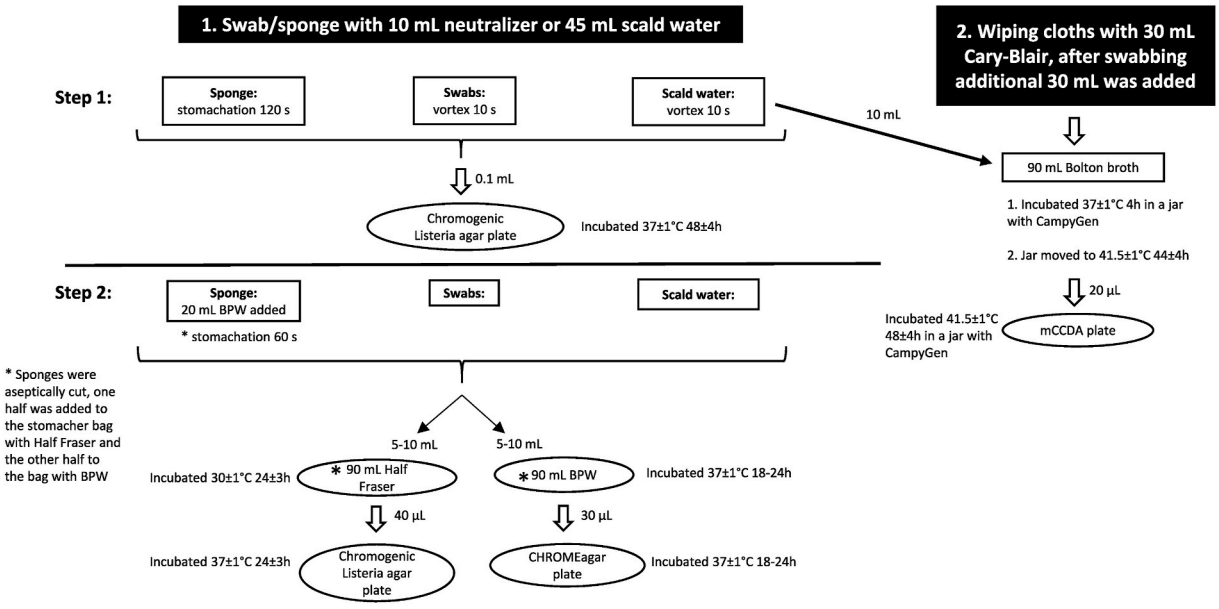

Fig. 1. Flow chart of analyses of *L. monocytogenes*, ESBL *E. coli,* and *Campylobacter* spp. BPW = buffered peptone water.

cultured on blood agar and incubated at 37 ± 1 °C for 24 ± 3 h, and then identified to species level with MALDI-TOF.

For analysis of ESBL *E. coli,* 5–10 mL of the dilution were added together with the other half of the sponge to a stomacher bag containing 90 mL BPW. From the original swab sample and scald water suspensions, 5–10 mL were added to 90 mL BPW and incubated at 37 ± 1 °C for 18–24 h. Then 30 µL were surface-plated on CHROM-agar Orientation (Chromagar, Paris, France), to which 1 mg/L cefotaxime had been added, and incubated at 37 ± 1 °C for 18–24 h (Fig. 1). After incubation for 18–24 h, characteristic colonies were selected for further re-culturing on blood agar and incubated at 37 ± 1 °C for 24 ± 3 h. Identification to species level was performed using MALDI-TOF.

2.5. Antimicrobial susceptibility testing

Testing for antimicrobial resistance (AMR) was performed on 11 *C. jejuni,* five *C. coli,* and one *E. coli* isolate. Susceptibility to selected antimicrobial substances was assessed with a Thermo Scientific™ Sensititre™ EUCAMP2 plate (for *Campylobacter* spp.) and Thermo Scientific™ Sensititre™ EUVSEC plate (for *E. coli*) (Thermo Fisher Scientific, Waltham, MA, USA). The reference strains *C. jejuni* (Culture Collection University of Gothenburg (CCUG) 33560) and *E. coli* (CCUG 17620) were used as a quality control for the EUCAMP2 and EUVSEC plate, respectively. Minimum inhibitory concentrations (MIC) of antimicrobials were determined by broth microdilution following the manufacturer's instructions and epidemiological cut-off (ECOFF) values for determining susceptibility were obtained from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) https://www.eucast.org/mic_and_zone_distributions_and_ecoffs/(*C. jejuni, C. coli,* and *E. coli*). No cut-off MIC value was available for sulphamethoxazole. Multidrug resistance was defined as resistance to three or more antibiotic classes. However, for aminoglycosides each substance was considered separately, because of the complexity of microbial resistance mechanisms to this class (Swedres-Swarm, 2021).

2.6. Whole-genome sequencing of bacterial isolates

A total of 32 bacterial isolates (17 *C. jejuni/C. coli* and 15 *L. monocytogenes*) were subjected to whole-genome sequencing (WGS) using Illumina technology. Bacterial genomic DNA was extracted and purified using an EZ1&2 DNA Tissue Kit (Qiagen, Hilden, Germany) together with an EZ1 Advanced XL instrument and a program card for bacteria (Qiagen), according to the manufacturer's recommendations. DNA concentrations were measured using a Qubit dsDNA Broad Range Assay Kit and a Qubit 4.0 instrument (Invitrogen, Carlsbad, CA, USA), and were standardized in DNase- and RNase-free water (Sigma-Aldrich, St Louis, MO, USA). Libraries were prepared using a Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. Normalization of libraries was performed manually in 10 mM Tris-HCl, pH 8.5 with 0.1% Tween 20 using the concentrations from a Qubit dsDNA High Sensitivity Kit and a Qubit 2.0 instrument (Invitrogen) and the average library size from a High Sensitivity DNA ScreenTape Analysis D1000 Kit (Agilent Technologies, Santa Clara, CA, USA) and a 4150 TapeStation (Agilent Technologies). Sequencing was performed on a NextSeq 500 system (Illumina) generating 2 × 150-bp paired-end reads.

All analyses of sequence reads obtained were performed in SeqSphere^{+} v. 8.5.1 (Junemann et al., 2013) (Ridom GmbH, Münster, Germany). Sequence read quality and adapter content were evaluated by FastQC v. 0.11.7 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/), while Trimmomatic v. 0.36 (Bolger et al., 2014) was used to remove any adapter sequences. Assembly was performed *de novo* using SKESA v. 2.4.0 (Souvorov et al., 2018) with default settings, which included downsampling to 180 × coverage and assembly remapping and polishing by BWA-MEM (Li, 2013). Mash Screen v. 2.1 (Ondov et al., 2019) was used to check the assemblies for possible contamination. Multilocus sequence typing (MLST) was carried out using existing schemes for *C. jejuni/C. coli* (Dingle et al., 2001) and *L. monocytogenes* (Ragon et al., 2008), and the Bacterial Isolate Genome Sequence database (BIGSdb) (Jolley et al., 2018). Core genome MLST (cgMLST) was performed using the respective schemes for *C. jejuni/C. coli* and *L. monocytogenes*, available at https://www.cgMLST.org. The cgMLST scheme for *C. jejuni/C. coli* contained 637 target loci (https://www.cgmlst.org/ncs/schema/145039/locus/) and a cluster distance threshold of 13, while the cgMLST scheme for *L. monocytogenes* contained 1701 target loci (https://www.cgmlst.org/ncs/schema/690488/locus/) and used 10 as cluster distance threshold. Minimum spanning trees (MST) to visualize the cgMLST data were created in SeqSphere+ (Ridom, GmbH), ignoring missing alleles in the pairwise comparisons. Identification of genes and/or point mutations causing AMR was performed using BLASTX search in NCBI AMRFinderPlus v.3.11.2 (Feldgarden et al., 2019). *Campylobacter jejuni* isolates of ST257 in this

study were compared to selected *C. jejuni* ST257 isolates from previous studies conducted in Sweden (Frosth et al., 2020; Hansson et al., 2020; Hansson et al., 2021a,b).

3. Results

In total, 484 samples were collected before ($n = 242$) and after ($n = 242$) C&D procedures. In general, each sampling point was sampled on 4–6 occasions before and after C&D, respectively. Not all sampling points could be sampled on all occasions, e.g., when the cleaning staff started to clean earlier than planned. Sampling points categorized as "Other" were only sampled on the first sampling occasion (Table 2). In both slaughterhouses, most surfaces were visually clean after C&D, but traces of feces, feathers, meat, fat, etc. were observed on some surfaces. Most surfaces were wet at the time of sampling, especially after C&D. All samples had a temperature of 2–8 °C on arrival at the laboratory. Two drains (sampling points 5 and 21, located in the red meat and poultry slaughterhouses, respectively) were the only sites where both *Campylobacter* spp. and *L. monocytogenes* were detected (Table 3).

3.1. Campylobacter spp

3.1.1. Red meat slaughterhouse

In the red meat slaughterhouse, *Campylobacter* spp. were detected in both FCS and NFCS samples taken before C&D in the slaughter area on five of six sampling occasions (4, 6, 8, 10, and 11) (Table 3). *Campylobacter* spp. were isolated from eight (13.0%) of 62 samples taken before C&D. Three *Campylobacter* species were identified: *C. coli* ($n = 5$) was most commonly isolated, followed by *C. jejuni* ($n = 3$) and *C. hyointestinalis* ($n = 1$). In one sample taken from the table for cattle organs, both *C. jejuni* and *C. hyointestinalis* were isolated (Table 3). *Campylobacter jejuni* ST58 isolates with indistinguishable cgMLST profiles were identified on the organ table and the cutting blade for carcasses during the same sampling occasion (sampling occasion 11). The other isolates were of different STs (ST828, ST1450, ST1470, ST6580, ST6585) (Fig. 2). After C&D, *Campylobacter* spp. were not detected in any of the samples.

3.1.2. Poultry slaughterhouse

In the poultry slaughterhouse, *Campylobacter* spp. were detected on FCSs and on NFCSs before C&D in both the slaughter and processing areas on two sampling occasions (2 and 3). In total, *Campylobacter* spp. were isolated from nine (15.5%) of 58 samples before C&D. The only species identified was *C. jejuni*, which was detected at least once at 80% (8/10) of the sampling points, not counting "Other" (Table 3). All seven isolates from sampling occasion 3 had indistinguishable cgMLST profiles (ST257). When comparing the *C. jejuni* isolates of ST257 from the present study with ST257 from other studies conducted in Sweden, the isolates from the poultry slaughterhouse were most closely related to three samples of calve feces from one dairy farm (4 alleles difference). The cluster also include eight isolates from chicken cecal samples from three different chicken producers and two isolates from fecal samples from another dairy farm (Fig. 3). The remaining two isolates from the poultry slaughterhouse were of different sequence types (ST19, ST1525), although sampled on the same occasion (Fig. 2). *Campylobacter* spp. were not detected after C&D in any of the samples.

3.1.3. Antimicrobial resistance in Campylobacter spp.

Of the five *C. coli* isolates detected in the red meat slaughterhouse (sampling occasions 4, 6, 8, and 11), which belonged to five different STs (828, 1450, 1470, 6580, 6585), four showed phenotypic resistance to streptomycin. These isolates were also the only *Campylobacter* isolates carrying the *aadE-Cc* gene. One *C. jejuni* isolate of ST19 detected in the drain of the processing area in the poultry slaughterhouse showed phenotypic resistance to ciprofloxacin and nalidixic acid, and had a mutation of T861 in the DNA gyrase (*gyrA*) gene. All other *Campylobacter* isolates from both slaughterhouses were phenotypically sensitive to all six antibiotics tested (Table 4). Four *C. coli* isolates without phenotypic resistance had the mutation *50S_L22_A103V* and one *C. coli* isolate without phenotypic resistance to quinolones had the mutation of T861 in *gyrA*. No bacterial growth was observed on the MIC plate of the sample containing both *C. jejuni* and *C. hyointestinalis*, so its AMR value could not be determined. None of the *Campylobacter* isolates showed multi-drug resistance.

3.2. Listeria monocytogenes

All L. *monocytogenes* isolates from both slaughterhouses, which belonged to ST7, ST8, and ST9, were from clonal complex (CC) 7, CC8, and CC9, respectively.

3.2.1. Red meat slaughterhouse

In the red meat slaughterhouse, *L. monocytogenes* was detected in eight (12.5%) of the 64 samples collected before C&D and in one sample (1.6%) collected after C&D (Table 3). *Listeria monocytogenes* was mainly present in drains, on five of the six occasions when *L. monocytogenes* was detected, and could be enumerated in two samples (3.1 and 1.0 log CFU/100 cm^2) before and in one sample (1.0 log CFU/100 cm^2) after C&D. In the drain in the slaughter area (sampling point 5), five different STs were identified (ST7, ST8, ST9, ST207, ST451) while in the drain in the processing area (sampling point 11) the isolates were of ST9. All six L. *monocytogenes* belonging to ST9 had indistinguishable cgMLST profiles although they were isolated on three separate sampling occasions 15 weeks apart (drains in the slaughter and processing area, and the cutting board in the processing area) (Fig. 4). In one drain, isolates of ST8 were detected on two different sampling occasions nine weeks apart. In one of the samples from drains, *L. monocytogenes* isolates identified in qualitative and quantitative analysis were of different STs (ST7 and ST9). Ten (83.3%) of the 12 isolated *L. monocytogenes* were of serogroup IIa (serotype 1/2a and 3a), while the others belonged to serogroup IIc (serotype 1/2c and 3c).

3.2.2. Poultry slaughterhouse

In the poultry slaughterhouse, *L. monocytogenes* was detected in three (5.2%) of 58 samples taken before C&D, all in qualitative analyses, but at levels below the limit of quantification. *Listeria monocytogenes* was not detected in any of the samples collected after C&D (Table 3). There was no close relationship between *L. monocytogenes* isolates, which were of STs 7, 8 and 504 (Fig. 4), but they were all of serogroup IIa (serotype 1/2a and 3a).

3.3. ESBL-producing E. coli

Presence of ESBL *E. coli* was not detected in the present study. One *E. coli* isolate was identified in a sample from the plucking fingers after C&D, but was sensitive to all antibiotics tested (sulfamethoxazole, trimethoprim, ciprofloxacin, tetracycline, meropenem, azithromycin, nalidixic acid, cefotaxime, chloramphenicol, tigecycline, ceftazidime, colistin, ampicillin and gentamicin).

4. Discussion

The analyses performed in this study showed that the C&D procedure used efficiently removed/inactivated *Campylobacter* spp. and *L. monocytogenes* from surfaces in the two slaughterhouses. Even on the sampling occasion on which *C. jejuni* was detected on almost all surfaces in the poultry slaughterhouse before C&D, *C. jejuni* was not detected in the samples taken after C&D. However, other studies have reported *Campylobacter* survival after C&D in poultry slaughterhouses (García-Sánchez et al., 2017; Peyrat et al., 2008). It should be noted that in this study, *Campylobacter* spp. were detected in a smaller number of samples before C&D than in other studies. The low number of samples

Table 3
Number of samples taken and identification of sampling points where *Campylobacter* spp., *Listeria monocytogenes* and extended-spectrum beta-lactamase *Escherichia coli* (ESBL *E. coli*) were isolated before and after cleaning and disinfection (C&D) in the two slaughterhouses. Superscript numbers indicate the sampling occasions (1-12) on which the bacteria were detected.

Slaughter-house	Area	Sampling point		C&D	Total No. of samples for *Campylobacter* spp.	*C. jejuni*	*C. coli*	*C. jejuni and C. hyointestinalis*	Total No. of samples for *L. monocytogenes* and ESBL *E. coli*	*L. monocytogenes*	ESBL *E. coli*
Red meat	**Slaughter**	**1**	Post-dehairing table pigs[a]	Before	6	–	–	–	6	–	–
				After	6	–	–	–	6	–	–
		2	Scald water pigs[a]	Before	4	–	–	–	4	–	–
				After	4	–	–	–	4	–	–
		3	Table cattle organs (evisceration)	Before	5	**1 (10)**	–	**1 (11)**	5	–	–
				After	5	**-**	–	**-**	5	–	–
		4	Conveyor belt pig organs (evisceration)	Before	5	–	**2 (6,8)**	–	6	–	–
				After	5	–	**-**	–	6	–	–
		5	**Drain cattle (carcass inspection)**	Before	5	–	**3 (4,6,11)**	–	6	**5 (4,6,8,10,11)**	–
				After	5	–	**-**	–	6	**-**	–
		6	Cutting blade cattle/pig (carcass splitting)[a]	Before	5	**1 (11)**	**-**	–	5	–	–
				After	5	**-**	**-**	–	5	–	–
		Other		Before	2	**-**	**-**	–	2	–	–
				After	2	–	–	–	2	–	–
	Processing	**7**	Cutting board beef cuts[a]	Before	6	–	–	–	6	**1 (8)**	–
				After	6	–	–	–	6	**-**	–
		8	Conveyor belt beef cuts[a]	Before	6	–	–	–	6	–	–
				After	6	–	–	–	6	–	–
		9	Conveyor belt packaging beef cuts[a]	Before	6	–	–	–	6	–	–
				After	6	–	–	–	6	–	–
		10	Trolley beef cuts[a]	Before	6	–	–	–	6	–	–
				After	6	–	–	–	6	–	–
		11	Drain (below cutting board)	Before	6	–	–	–	6	**2 (8,11)**	–
				After	6	–	–	–	6	**1 (6)**	–
Poultry	**Slaughter**	**12**	Cutting blade bleeding[a]	Before	5	**1 (3)**	**-**	–	5	–	–
				After	5	**-**	**-**	–	5	–	–
		13	Scald water[a]	Before	5	–	–	–	5	–	–
				After	5	–	–	–	5	–	–
		14	Plucking fingers[a]	Before	5	–	–	–	5	–	–
				After	5	–	–	–	5	–	–
		15	Shackle after stunning[a]	Before	5	**1 (3)**	**-**	–	5	–	–
				After	5	**-**	**-**	–	5	–	–
		16	Floor lairage	Before	5	**1 (3)**	**-**	–	5	–	–
				After	5	**-**	**-**	–	5	–	–
		Other		Before	5	**-**	**-**	–	5	**1 (2)**	–
				After	5	–	–	–	5	–	–
	Processing	**17**	Conveyor belt chicken cuts[a]	Before	5	**1 (3)**	**-**	–	5	–	–
				After	5	**-**	**-**	–	5	–	–
		18	Conveyor belt chicken cuts[a]	Before	6	**1 (3)**	**-**	–	6	–	–
				After	6	**-**	**-**	–	6	–	–
		19	Cutting blade thighs[a]	Before	6	**1 (3)**	**-**	–	6	–	–
				After	6	**-**	**-**	–	6	–	–
		20	Salt injector needles[a]	Before	5	**1 (3)**	**-**	–	5	–	–
				After	5	**-**	**-**	–	5	–	–
		21	**Drain (below cutting blade thighs)**	Before	6	**2 (2,3)**	**-**	–	6	**2 (5,7)**	–
				After	6	**-**	**-**	–	6	**-**	–

[a] food contact surfaces.

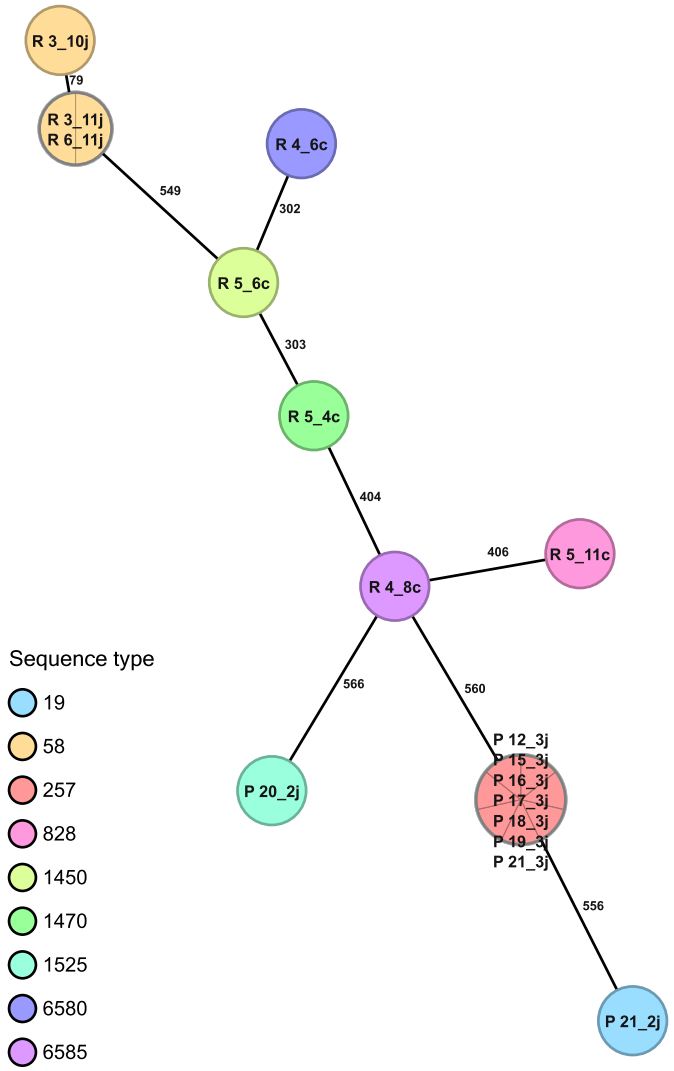

Fig. 2. Minimum spanning tree of core genome multi-locus sequence typing (cgMLST) data from *Campylobacter* spp. isolated from the red meat (R) and poultry (P) slaughterhouse ($n = 17$) (first value after slaughterhouse type (R/P) indicates sampling point, second value indicates sampling occasion). j = *C. jejuni*. c = *C. coli*. Values on lines are number of allelic differences (line length not proportional to number). Grey rings indicate genotypes belonging to the same MST cluster. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

with *Campylobacter* was most likely due to low (around 5%) *Campylobacter* prevalence in chickens in Sweden during the study period (National Veterinary Institute, 2022), while most other countries have a significantly higher prevalence of *Campylobacter* in their chickens (EFSA and ECDC, 2022). *Campylobacter jejuni* was the only *Campylobacter* species detected in the poultry slaughterhouse, confirming previous findings that it is the most commonly detected *Campylobacter* species in poultry (Hansson et al., 2021a; Hauge et al., 2023; Marotta et al., 2015; Moazzami et al., 2021).

Campylobacter isolates, which were indistinguishable based on their cgMLST profiles, were detected at multiple sampling points in both slaughterhouses before C&D. Notably, many of these sampling points were FCS such as the cutting blades in both slaughterhouses (sampling points 6, 12, 19), as well as the conveyor belts for chicken cuts and salt injector needles in the cutting area in the poultry slaughterhouse. This raises concerns about the potential risk of meat cross-contamination during production. In the poultry slaughterhouse in particular, *C. jejuni* strain ST257 showed great potential to spread to many different sampling points in both the slaughter and processing areas. This is a hygiene challenge for food business operators, since the STs detected in the poultry slaughterhouse (ST257 and ST19) are known to cause human outbreaks and have been detected in chicken meat in Sweden (Swedish Food Agency and Public Health Agency of Sweden, 2018; Public Health Agency of Sweden, 2020). The *C. jejuni* ST257 isolates were compared to those from other studies conducted in Sweden within a similar time frame. From this comparison, fecal samples from cattle collected in a different geographic region and time period were found to be related to the *C. jejuni* isolates from the present study, complicating the interpretation of the relationship between these isolates. One possible explanation is that this sequence type is common and may have been transferred through wild birds. Another possibility is that the isolates do not share a common source, and differences in accessory genes were not considered in the analysis, since only the core genome was analyzed.

An AMR study of the *Campylobacter* isolates detected in this study was performed to compare the occurrence of resistance against important antibiotics that are included in the national surveillance program on AMR in Sweden. Phenotypic and genotypic quinolone resistance was observed for one *C. jejuni* isolate from the poultry slaughterhouse. *Campylobacter* spp. with quinolone resistance have previously been detected in poultry and poultry meat in Sweden and other European countries (García-Sánchez et al., 2017; Hansson et al., 2021b; Torralbo et al., 2015). The occurrence of resistance to fluoroquinolones was 20% in Swedish broilers in 2022 (Swedres-Svarm, 2022). This is higher than the occurrence found in the present study (9%), but the difference could be due to the low number of *Campylobacter* isolates analyzed. The reason for the resistance detected in the present study is unknown, but is unlikely to be due to antimicrobial use, since chickens are not treated with quinolones and are overall rarely treated with antimicrobials in Sweden nowadays (Hansson et al., 2021b; T. Dzieciolowski, personal communication September 19, 2023).

Remarkably high (80%) phenotypic and genotypic streptomycin resistance was observed in the *C. coli* isolates detected in the red meat slaughterhouse. It could not be established whether these isolates originated from pigs or cattle, since both species were slaughtered in the same facility. *Campylobacter coli* is known to be the most common *Campylobacter* species in pigs, whereas in cattle *C. coli* is much less frequently occurring than *C. jejuni* (Hansson et al., 2021b; Swedres-Swarm, 2015; Thépault et al., 2018). At European level, *C. coli* resistance to streptomycin is high in both pigs and calves (EFSA-ECDC, 2023). In Sweden, there is high (47%) phenotypic streptomycin resistance in *C. coli* from pigs and half of resistant isolates are also sensitive to other antimicrobials (Swedres-Swarm, 2019), which agrees with findings in the present study. This resistance to streptomycin is difficult to explain by selection pressure, since this compound is rarely used in Sweden nowadays (Swedish Medical products Agency, 2013; Swedres-Swarm, 2019). A possible explanation could be the mutable nature of *Campylobacter*, which had developed several mechanisms for antibiotic resistance, including point mutations, acquisition of resistance genes, and efflux systems (Wirz et al., 2010). This poses a threat to human health, since AMR in animals and humans is linked, and may lead to difficulty in treating infections (EFSA-ECDC, 2023).

Listeria monocytogenes was found in drains in both slaughterhouses on several sampling occasions. Interestingly, *L. monocytogenes* was more frequently found in the drain in the slaughter area in the red meat slaughterhouse than in the two drains in the processing areas in both slaughterhouses. A possible reason could be that surfaces in slaughter areas generally gets more soiled than in processing areas. As observed for *Campylobacter*, the C&D procedure removed *L. monocytogenes* from these sampling points. However, on one sampling occasion, *L. monocytogenes* was detected in a drain in the processing area in the red meat slaughterhouse despite C&D. This is not surprising, since *L. monocytogenes* is commonly found in drains in food processing plants (Muhterem-Uyar et al., 2015). Isolates of L. *monocytogenes* ST9 with

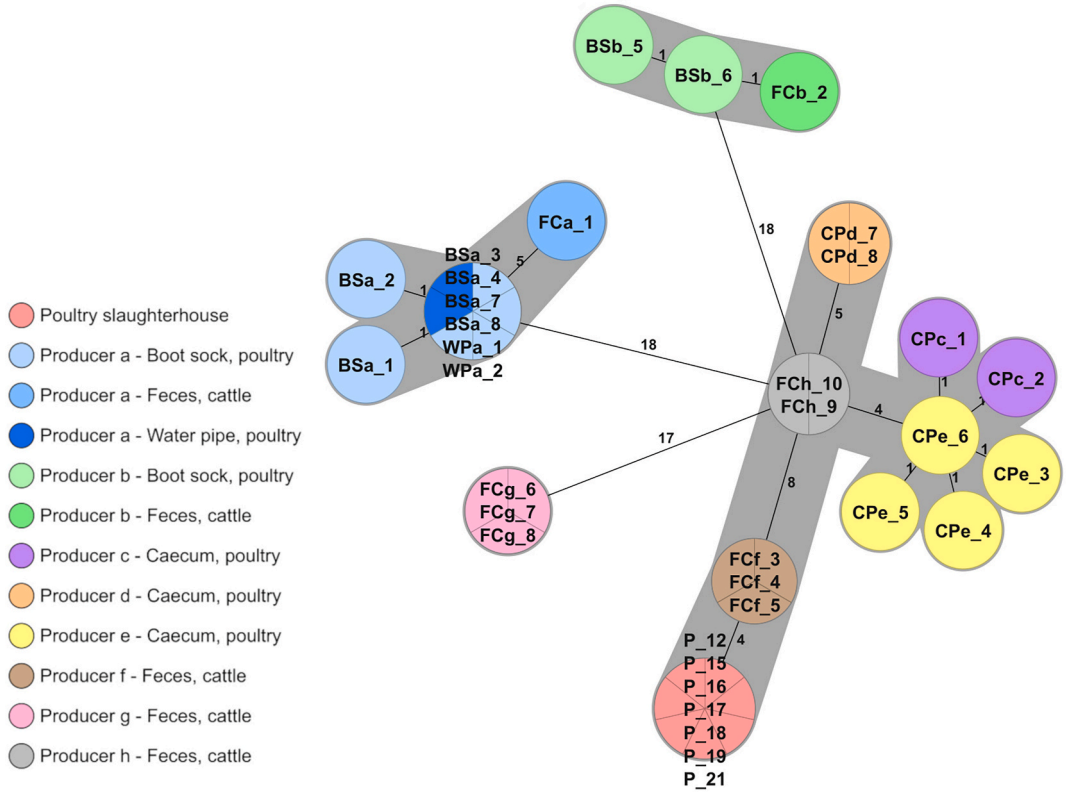

Fig. 3. Minimum spanning tree of core genome multi-locus sequence typing (cgMLST) data from *Campylobacter jejuni* of sequence type 257 isolated from the poultry (P) slaughterhouse ($n = 7$) in the present study and, samples from poultry caeca ($n = 8$), fecal samples from cattle ($n = 10$), samples from boot socks ($n = 8$) and water pipes ($n = 2$) from other studies. Values on lines are number of allelic differences (line length not proportional to number). Grey zones indicate genotypes belonging to the same MST cluster.

Table 4
Distribution of minimum inhibitory concentrations (MICs, mg/L) and antibiotic resistance (Res %) in *Campylobacter jejuni* ($n = 11$) and *C. coli* ($n = 5$) isolates, expressed as number of isolates at different MIC values. White fields denote range of diluents tested for each antibiotic and vertical bold lines indicate cut-off values used to define resistance. MIC values equal to or lower than the lowest concentration tested are given as the lowest concentration tested.

Antibiotic	*Campylobacter* spp.	Res (%)	≤0.12	0.25	0.5	1	2	4	8	16	32	64	128
Ciprofloxacin	*C. jejuni*	1/11 (9.1)	10						**1**				
	C. coli	0/5 (0)	5										
Erythromycin	*C. jejuni*	0/11 (0)				11							
	C. coli	0/5 (0)				5							
Gentamicin	*C. jejuni*	0/11 (0)	2	1	8								
	C. coli	0/5 (0)			3	2							
Nalidixic acid	*C. jejuni*	1/11 (9.1)					8	1	1			**1**	
	C. coli	0/5 (0)						5					
Streptomycin	*C. jejuni*	0/11 (0)			1	1	9						
	C. coli	4/5 (80)						1		**4**			
Tetracycline	*C. jejuni*	0/11 (0)			11								
	C. coli	0/5 (0)			5								

indistinguishable cgMLST profiles were found in the processing area of the red meat slaughterhouse on three sampling occasions 15 weeks apart. On one of these sampling occasions, this strain was detected after C&D, while on the other two occasions it was detected before C&D. There are possible explanations for this, e.g., the same strain may have been re-introduced several times from pigs originating from the same farms or the strain may have persisted in the slaughterhouse, but was not detected after C&D due to e.g., relatively small sampling area. A limitation in the present study was that the maximum sampled area was 100 cm^2. To ensure that no pathogens are present, a considerably larger area should be sampled (≥1000 cm^2) (Carpentier and Barre, 2012; Swedish Standards Institute, 2018). Because this study was part of a larger study in which hygiene indicator bacteria were quantified (Moazzami et al., 2023), it was not feasible to sample a larger area.

Other studies have shown that *L. monocytogenes* ST9 and ST121 are the most common *L. monocytogenes* sequence types persisting in the food industry and that ST9 in particular is associated with persistence in the meat industry in several European countries, including Sweden's neighboring country Norway (Fagerlund et al., 2020; Melero et al., 2019). Clonal complexes 7 and CC8 have previously been reported as the

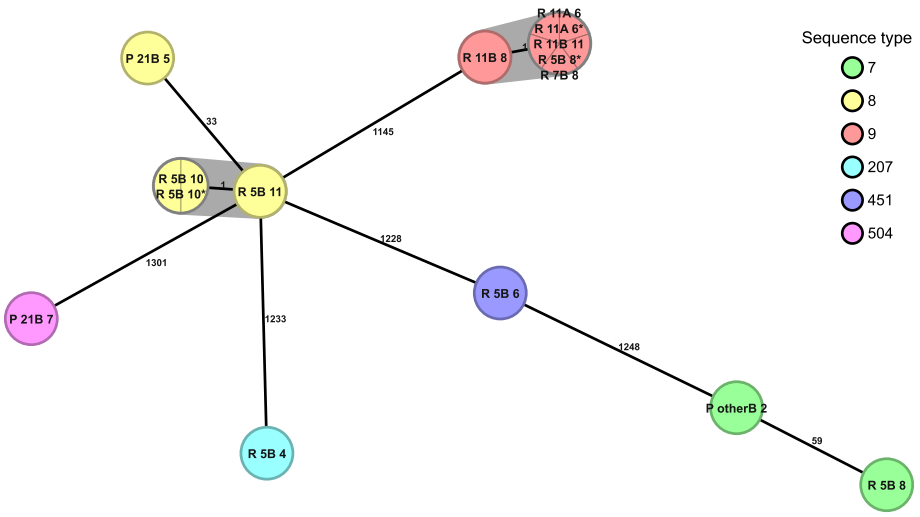

Fig. 4. Minimum spanning tree (MST) of core genome multi-locus sequence typing (cgMLST) data from *Listeria monocytogenes* isolated from the red meat (R) and poultry (P) slaughterhouses before (B) and after (A) cleaning and disinfection ($n = 15$) (first value after slaughterhouse type (R/P) indicates sampling point, second value indicates sampling occasion). * indicates detection in quantitative analysis. Values on lines are number of allelic differences (line length not proportional to number). Grey zones indicate genotypes belonging to the same MST cluster. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

most commonly found CCs in Sweden (Møretrø et al., 2024). Clonal complexes 7, CC8, and CC9 are also among the most common CCs in other European countries (Fagerlund et al., 2022; Møretrø et al., 2024; Stoller et al., 2019). Moreover, when *L. monocytogenes* is found in drains, it has the potential to contaminate nearby FCS (Saini et al., 2012). It is worrisome that *L. monocytogenes* was detected on two FCS before C&D, and similar recovery of this bacterial species on FCS in poultry and red meat slaughterhouses has been reported previously (Gómez et al., 2015; Martín et al., 2014; Muhterem-Uyar et al., 2015). As with *Campylobacter*, this poses a risk of cross-contamination of the meat (Martín et al., 2014).

Listeria monocytogenes isolates of ST8 and ST9, serogroups and serotypes identified in the present study have been found to cause human disease and outbreaks in Sweden and other parts of Europe, and some of these have been found on meat processing surfaces, raw meat, and meat products such as RTE (EFSA Panel on Biological Hazards, 2018; Martín et al., 2014; Lindblad and Flink, 2017; M. Ricao, National Food Agency, personal communication, March 7, 2023; Okpo et al., 2015; Ottoson, 2019). This shows the importance of controlling this hazard at slaughterhouse level by applying adequate C&D procedures and a hygienic slaughter process.

Campylobacter spp. and *L. monocytogenes* were not isolated from scald water either before or after C&D in the two slaughterhouses. This could be due to the low volume of scald water analyzed in this study, or it could indicate that viable bacteria of these pathogens were not present (Osiriphun et al., 2012). Other studies conducted in poultry slaughterhouses have detected *Campylobacter* spp. in scald water before C&D (Torralbo et al., 2015) and after C&D (Peyrat et al., 2008). The temperature of the scald water could have resulted in survival of the bacteria in those studies, since it was 52 °C in the study by Torralbo et al. (2015), although it was not specified by Peyrat et al. (2008). In the present study, scald water temperature was approximately 60 °C and 54 °C just before C&D and 45 °C and 44 °C after C&D in the red meat and poultry slaughterhouse, respectively (Moazzami et al., 2023). Scald water temperature was similar (52–56 °C) in other studies in which *Campylobacter* spp. was not found in poultry slaughterhouses (Gruntar et al., 2015; Hauge et al., 2023; Perez-Arnedo and Gonzalez-Fandos, 2019).

Presence of ESBL *E. coli* was not detected in this study, in line with the decreasing prevalence of this pathogen in broilers, broiler meat and pig meat in Sweden and some other European countries in recent years (Althaus et al., 2017; EFSA-ECDC, 2023; Swedres-Swarm, 2021). However, it should be noted that on some CHROMagar plates, characteristic *E. coli* colonies were found in mixed culture. Re-culturing attempts were made for further identification, but it was not possible to obtain the bacteria in pure culture. Thus, it is possible that these bacteria were present in low concentration in some samples without being identified.

5. Conclusions

This study demonstrated that it is possible to remove pathogenic bacteria such as *Campylobacter* spp. and *L. monocytogenes* from surfaces in slaughterhouses and meat processing plants by proper cleaning and disinfection, which create important hurdles in control of foodborne pathogens. The serotypes, sequence types, and clonal complexes of *Campylobacter* spp. and *L. monocytogenes* isolates detected on surfaces in this study are known to cause human disease in Sweden and other European countries. Therefore correct sampling of surfaces in slaughterhouses and meat processing plants to detect these pathogens and, when necessary, improve the C&D procedure is fundamental for production of safe meat. This study also demonstrated that failure of the hygiene process in slaughterhouses is associated with increased risk of cross-contamination of meat during slaughter.

CRediT authorship contribution statement

Madeleine Moazzami: Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Emma Bergenkvist:** Writing – review & editing, Investigation. **Sofia Boqvist:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Sara Frosth:** Writing – review & editing, Methodology, Investigation, Funding acquisition. **Solveig Langsrud:** Writing – review & editing. **Trond Møretrø:** Writing – review & editing. **Ivar Vågsholm:** Writing – review & editing, Funding acquisition, Conceptualization. **Ingrid Hansson:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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