

DATA ARTICLE

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Assessment of beef carcass contamination with *Salmonella* and *E. coli* O 157 in slaughterhouses in Bishoftu, Ethiopia

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Abstract

Background: *Salmonella* and *E. coli* O157 are common causes of foodborne diseases. Evisceration and de-hiding steps can lead to carcass contamination during slaughter operation. In Ethiopia, information on the association between the presence of these pathogens in the rectal content and/or on the hide of cattle and their presence on the carcass is lacking.

Methods: The aim of this study was to assess the sources of beef carcass contamination with *Salmonella* and *E. coli* O157 during slaughter. Rectal contents and hide- and carcass-swabs (from three sites: foreleg, brisket and hind leg) were collected from 70 beef cattle at two small scale slaughterhouses. Isolates were genotyped by the Pulsed Field Gel Electrophoresis method and tested for resistance against 14 microbial drugs.

Results: *Salmonella* was detected at equal proportions (7.1%) in rectal content samples and hide swabs. *E. coli* O157 was detected in 8.6% of the rectal contents and 4.3% of the hide swabs. The proportion of contaminated carcasses was 8.6% for *Salmonella* and 7.1% for *E. coli* O157. Genetic linkage between the *Salmonella* and *E. coli* O157 isolates from the rectal contents and/or hides and carcasses were observed only in a few cases (2 and 1 carcasses, respectively) indicating the limited direct transfer of the pathogens from the feces and/or hide to the carcass during slaughter. Most carcasses became positive by cross contamination. All the *S. Typhimurium* isolates ($n = 8$) were multidrug resistant being resistant to ampicillin, chloramphenicol, sulfamethoxazole and tetracycline. The two *S. Dublin* isolates were resistant to colistin. All *E. coli* O157 isolates were susceptible to the antimicrobials tested.

Conclusion: The results indicated that cross contamination may be an important source for carcass contamination.

Keywords: *Salmonella*, *Escherichia coli* O157, Slaughterhouse, Beef carcass, Contamination, Rectal content, Hide

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Introduction

Foodborne diseases (FBD) are a worldwide problem. Consumption of contaminated food of animal origin is associated with potential food safety risks and a major source of FBD. *Salmonella* and Shiga toxin-producing *E. coli* are major causes of FBD (Havelaar et al. 2015). Ruminants, particularly cattle, are reservoirs and asymptomatic carriers of *Salmonella* (Cummings et al. 2010; Gutema et al. 2019) and *E. coli* O157 (Gyles 2007). Studies reported the occurrence of these pathogens in the feces and on the hides of cattle on farms and in slaughterhouses in developed countries (Arthur et al. 2010; Cobbaut et al. 2008; Essendoubi et al. 2019; Madoroba et al. 2016). The presence of *Salmonella* and *E. coli* O157 in the feces and on the hides of cattle may lead to their transfer to carcasses during hide removal and evisceration (Croxen et al. 2013; Cummings et al. 2010; Gutema et al. 2021a).

Consumption of contaminated beef and beef products is one of the transmission routes of *Salmonella* and *E. coli* O157 to humans (EFSA and ECDC 2018; Pires et al. 2019) and has been implicated in many foodborne outbreaks (CDC 2016; Plumb et al. 2019). This is particularly important in countries like Ethiopia where consumption of raw or under-cooked beef in the form of steak (“*kurt*”) or beef tartare (“*kitfo*”) made from raw minced beef, is common (Avery 2014; Seleshe et al. 2014). Consumption of raw beef products can be a source of *Salmonella* and *E. coli* O157 infections in Ethiopia (Gutema et al. 2021b, c).

In Ethiopia, few studies have reported the prevalence of *Salmonella* in cattle feces (Gutema et al. 2021c; Takele et al. 2018), on hides (Sibhat et al. 2011) and on carcasses (Atsbha et al. 2018; Takele et al. 2018). Similarly, *E. coli* O157 was reported in cattle feces (Abdissa et al. 2017; Gutema et al. 2021b; Haile et al. 2017), on hides (Abdissa et al. 2017) and on carcasses (Atnafie et al. 2017) at the slaughterhouse level. We previously identified dehiding and evisceration as two major potential sources of carcass contamination at slaughterhouses in Ethiopia (Gutema et al. 2021a). However, there is currently no data confirming the potential association between the presence of these pathogens in the rectal content and/or on the hide of cattle and their presence on the carcass. Determining the genetic relatedness of *Salmonella* and *E. coli* O157 in cattle feces, on the hide and on the carcass is essential to investigate the potential transfer to carcasses. This will also contribute to the identification of critical control points and the development of mitigation strategies to ensure beef safety.

The objective of this study was to investigate the occurrence and the genetic relatedness for both *Salmonella* and *E. coli* O157 isolated from the rectal content and hide, and the carcass at slaughterhouses. Antimicrobial

resistance of *Salmonella* and *E. coli* O157 isolates obtained from rectal contents, hides and carcass was further assessed.

Materials and methods

Slaughterhouses

The study was conducted from November 2018 to May 2019 at two slaughterhouses in Bishoftu town located in East Shoa Zone, Oromia, Ethiopia. Both slaughterhouses were small in process capacity whereby the municipal slaughterhouse and the private slaughterhouse usually slaughtered 5–15 and 15–30 cattle per day, respectively. The retail shop owners buy cattle from open markets and bring them to the slaughterhouse for slaughter service. The slaughter process at both slaughterhouses was rather similar. Briefly, the manual slaughter process involved stunning with a sharp knife, bleeding by cutting arteries and veins in the throat region, removal of head and feet and dehiding the upper part of the hind legs on the floor followed by hanging of the carcass, manual dehiding, evisceration, carcass washing, post-mortem inspection, carcass labelling and storage at environmental temperature until distribution to retail shops. The slaughterhouses did not have a stand-by pressurized water supply and hot water for hand and equipment including knives washing. Moreover, slaughterhouse workers were involved in different slaughter steps and received no or limited hygienic training (Gutema et al. 2021a).

Sample collection

Samples were collected from 70 animals (35 in each slaughterhouse). Seven visits per slaughterhouse were organized whereby each time, 5 carcasses were sampled during slaughter. Due to the presence of relatively many cattle in the lairage of the private slaughterhouse, animals were selected using systematic random sampling before slaughter whereas at the municipal slaughterhouse due to the limited number of animals present in the lairage, five consecutively slaughtered animals slaughtered the day of sampling were sampled. The following samples were collected from each carcass: one rectal content (50 g), one hide and three carcass swab samples. The hide swab was taken from the medial side of the foreleg and hind leg and the brisket from one half of the carcass immediately after stunning. From each hide swabbing site, an area of 20 × 20 cm was swabbed using the same sterile cotton swab pre-moistened in 10 ml buffered peptone water (BPW; Difco, BD, Sparks, MD, USA). Separate carcass swabs (20 × 20 cm) per site were obtained after evisceration and before washing from the same sites as the hide swabs, but on the other half of the carcass. Samples were transported in an

icebox to the laboratory and stored at 4 °C until processing within 24 h.

Detection of *Salmonella* and *E. coli* O157

For processing of the hide and carcass swabs, each swab in 10 ml BPW was transferred into a stomacher bag containing another 30 ml BPW to make a final volume of 40 ml and homogenized for 2 min using a stomacher. From the final volume of homogenized solution, 20 ml was transferred into another stomacher bag.

Salmonella detection was based on the International Organization for Standardization guideline ISO 6579-1: 2017 (ISO 2017). Briefly, 25 g of rectal content was transferred into a sterile stomacher bag, 225 ml of BPW was added and the mixture was homogenized using a stomacher blender for 1 min at 200 rpm. Homogenized rectal content, hide and carcass swabs were incubated at 37 °C for 18 h. After the incubation of the pre-enrichment broths, 0.1 ml of each culture medium was spotted in 3 drops onto a modified semi solid Rappaport-Vassiliadis medium (MSRV; Oxoid, Basingstoke, UK) and incubated at 41.5 °C for 24 h. After incubation, plates were examined for the presence of migration zones. A loopful from the edge of a migration zone was streaked onto xylose lysine deoxycholate (XLD, Difco) agar plates and incubated at 37 °C for 24 h. Plates were examined for the presence of suspect *Salmonella* colonies. Suspected colonies were biochemically tested using triple sugar iron agar slants (Difco, BD), lysine decarboxylase test (BBL, BD), and indole test (BBL, BD). One confirmed isolate per sample was stored at -18 °C for further characterization. Collected *Salmonella* isolates were subjected to a *S. Typhimurium* PCR using the primers described by Lin et al. (1999). All isolates negative for this PCR were then clustered using enterobacterial repetitive intergenic consensus (ERIC) PCR as described by Rasschaert et al. (2005). Based on the data obtained from each ERIC profile at least one isolate was selected for serotyping according to the Kauffmann-White scheme (Grimont and Weill 2007) at Belgian National Reference Laboratory for *Salmonella*.

E. coli O157 detection was based on International Organization for Standardization, horizontal method for the detection of *E. coli* O157-ISO 16654: 2001 (ISO 2001). Twenty-five gram of each rectal content sample was transferred into a stomacher bag containing 225 ml of modified tryptone soya broth (Oxoid) supplemented with 20 mg/l novobiocin (Sigma Aldrich, MO; USA) (mTSBn), homogenized using a stomacher blender for 1 min at 200 rpm. For the detection of *E. coli* O157 from the swab samples, 20 ml double concentrated mTSBn was added to stomacher bags containing 20 ml of the sample homogenate. After the incubation of the enrichment broths at 41.5 °C for 6 h, 1 ml of each broth was

manually subjected to immunomagnetic separation (IMS) using Dynabeads anti-*E. coli* O157 (ThermoFisher Scientific, West Palm Beach, FL, USA) according to the manufacturers' instruction. The final washed bead-bacteria complexes were spread onto cefixime tellurite sorbitol MacConkey agar plates (Oxoid) containing 0.05 mg/l cefixime and 2.5 mg /l potassium tellurite (Oxoid) (CT-SMAC). After incubation at 37 °C for 24 h, the plates were examined for the presence of suspect colonies. From each selective agar plate, up to three suspect colonies were subjected to Kligler Iron agar, indole and *E. coli* O157 latex agglutination (Oxoid) tests. In the frame of another research project, one isolate per positive sample was further analyzed using whole genome sequencing method described by De Rauw et al. (2019) at the Belgian National Reference Center for STEC. Data on the presence of *stx* genes, *eae* gene, and *ehxA* gene in those isolates was obtained from this analysis.

Pulsed field gel electrophoresis (PFGE)

Both *Salmonella* and *E. coli* O157 isolates (one isolate per positive sample) were genotyped by PFGE after digestion with *Xba*I enzyme (CDC 2017). The fingerprints were grouped according to their similarity with Bionumerics 7.6 software (Applied Maths, Biomérieux, Sint-Martens-Latem, Belgium) using the band-based dice coefficient with a 2% position tolerance and unweighted-pair group method using arithmetic averages (UPGMA). Pulsotypes were assigned based on the difference of at least one band in the fingerprints and indicated by capital letter.

Antimicrobial susceptibility testing

All *Salmonella* and *E. coli* O157 isolates were tested for their antimicrobial resistance to the following 14 antimicrobial drugs with tested concentration range (µg/ml) in brackets: ampicillin (1–64), azithromycin (2–64), cefotaxime (0.25–4), ceftazidime (0.5–8), chloramphenicol (8–128), ciprofloxacin (0.015–8), colistin (1–16), gentamicin (0.5–32), meropenem (0.03–16), nalidixic acid (4–128), sulfamethoxazole (8–1024), tetracycline (2–64), tigecycline (0.25–8) and trimethoprim (0.25–32). The resistance profiling was evaluated based on the minimum inhibitory concentration (MIC) using Sensititre EU surveillance *Salmonella*/*E. coli* (EUVSEC) plates (Thermo Fisher Scientific, Merelbeke, Belgium). The tests were performed according to the manufacturer's instructions. The standard reference strain *E. coli* ATCC 25922 was used as quality control. European Committee on Antimicrobial Susceptibility Testing (EUCAST) epidemiological breakpoint values were used to categorize the isolates as resistant or susceptible. In case of *Salmonella*, for sulfamethoxazole, tigecycline and colistin the

epidemiological breakpoints for *E. coli* were used (EUCAST 2019).

Results

From the 70 cattle examined, 23 (32.9%) were positive for *Salmonella* and/or *E. coli* O157 in at least one sample. Specifically, 14 (20.0%) animals were positive for *Salmonella*, and 11 (15.7%) for *E. coli* O157 (Table 1). Two animals were positive for both *Salmonella* and *E. coli* O157 (Table 2).

Salmonella

From the 14 *Salmonella* positive carcasses, the following 16 samples were positive: 5 rectal contents (7.1%), 5 hides (7.1%) and 6 carcasses (1 foreleg, 2 briskets and 3 hind legs) (8.6%). Only in two cases, two samples of the same animal were positive: the rectal content and the carcass (brisket) from one animal, and the rectal content and the carcass (hind leg) from the other animal. The 16 *Salmonella* isolates were identified as *S. Chailey*, *S. Dublin*, *S. Muenchen* and *S. Typhimurium*. All isolates within a serotype belonged to a single pulsotype (Fig. 1). The isolates from the animals with two positive samples were identified as *S. Typhimurium*. The supplementary Table 1 shows the MIC distributions of the *Salmonella* isolates. All *S. Typhimurium* isolates showed the same resistance profile, namely resistant to ampicillin, chloramphenicol, sulfamethoxazole and tetracycline, while the two *S. Dublin* isolates were only resistant to colistin. *Salmonella* Chailey and *S. Muenchen* were sensitive to all 14 antimicrobial drugs tested.

E. coli O157

Of the 11 *E. coli* O157 positive carcasses, the following 14 samples were positive: 6 rectal contents (8.6%), 3 hides (4.3%) and 5 carcasses (1 foreleg, 2 briskets and 2 hind legs) (7.1%). In three cases, two samples of the same animal were positive for *E. coli* O157: hide and carcass (hind leg), rectal content and hide, and rectal

content and carcass (hind leg). *E. coli* O157 isolates were grouped into eight pulsotypes (A-I) (Fig. 2). Among the isolates obtained from the same animals ($n = 3$), genetic relatedness was observed only between isolates obtained from a hide and a carcass (hind leg) swab of one animal sampled at the municipal slaughterhouse. All the *E. coli* O157 isolates carried the *eae* and the *ehxA* gene; the *stx2* gene (10 *stx2c* and 2 *stx2a*) was detected in 85.7% (12/14) of the isolates while the *stx1* gene was not detected in any of the isolates. The *stx2a* subtypes were detected in isolates from a brisket and a hide swab. All *E. coli* O157 isolates were sensitive to the 14 antimicrobial drugs tested.

Discussion

The present study detected for the first time in Ethiopia *Salmonella* and *E. coli* O157 in the rectal content, on the hide and on cattle carcass at slaughterhouses. Although *Salmonella* was detected at the same proportion (7.1%) of the feces and the hide swabs, it was not simultaneously detected from the same carcasses. The proportion of positive rectal contents was comparable with the national prevalence estimate of 7.1% (variation from 2.1% to 16.2%) in Ethiopia (Tadesse and Tessema 2014) but lower than the pooled prevalence estimate of 15.4% with a variation from 11.7 to 20% in Africa (Thomas et al. 2020). The proportion of *Salmonella* positive hide samples was lower compared to a study by Sibhat et al. (2011) who reported a prevalence of 31% in Ethiopia. Studies that have been conducted elsewhere indicated the occurrence of *Salmonella* on hides of cattle at slaughterhouses with a variable prevalence ranging from 17.7% in England (Reid et al. 2002) and up to 94% in USA (Brichta-Harhay et al. 2008).

The proportion of *E. coli* O157 in rectal contents and on hides was 8.6 and 4.3%, respectively. The proportion of positive rectal contents was slightly higher compared to global prevalence estimate of 5.7% that ranges from 0.1% to 61.8% (Islam et al. 2014). A recent study by

Table 1 Proportion of *Salmonella* and *E. coli* O157 in the rectal content, hide and carcass swabs obtained from 70 beef cattle in Bishoftu, Ethiopia

Source	Number of samples	<i>Salmonella</i>		<i>E. coli</i> O157
		Number (%)	Serotypes	Number (%)
Rectal content	70	5 (7.1)	Typhimurium (5)	6 (8.6)
Hide	70	5 (7.1)	Typhimurium (1), Dublin (1), Chailey (2), Muenchen (1)	3 (4.3)
Carcass	210	6 (8.6)		5 (7.1)
Fore leg	70	1 (1.4)	Dublin (1)	1 (1.4)
Hind leg	70	3 (4.3)	Typhimurium (1), Chailey (1), Muenchen (1)	2 (2.8)
Brisket	70	2 (2.8)	Typhimurium (1), Muenchen (1)	2 (2.8)
Total	350	16		14
Animal		14 (20.0)		11 (15.7)

Table 2 Distribution of *Salmonella* and *E. coli* O157 isolates among the positive cattle identified at two slaughterhouses in Bishoftu, Ethiopia

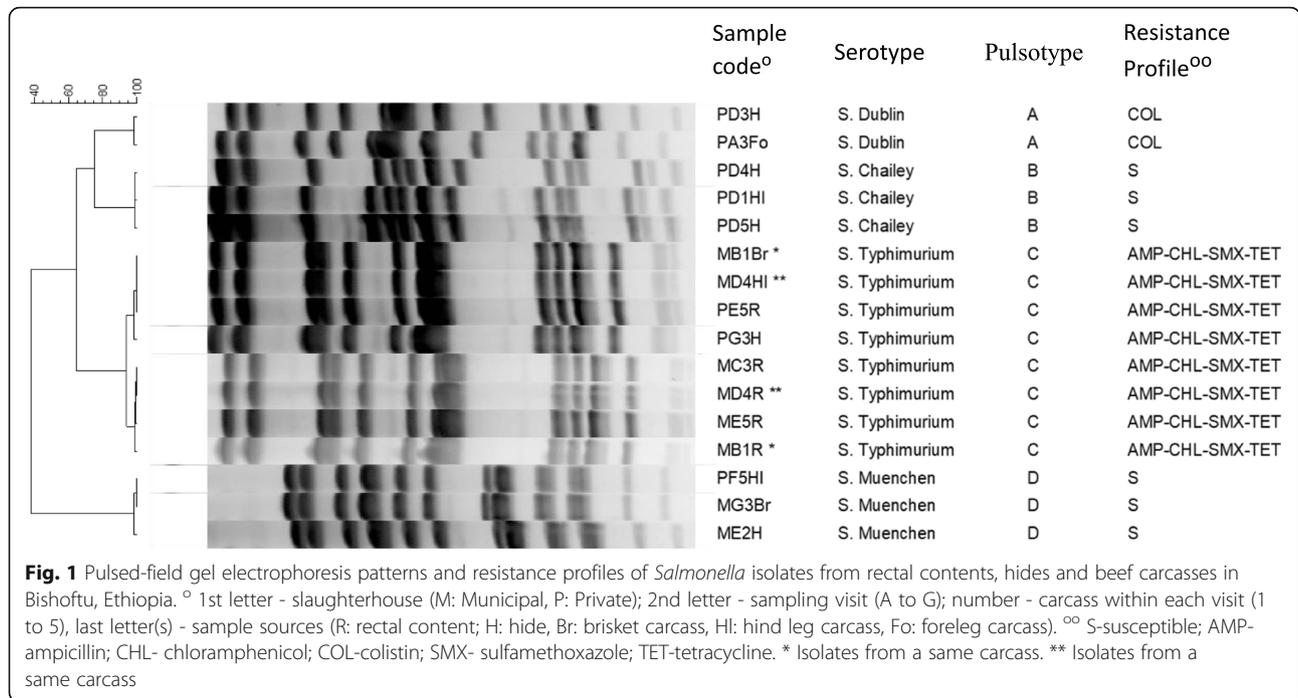
Slaughterhouse	Visit	ID Animal*	Sample type				
			Rectal content	Hide	Carcass swabs		
					Foreleg	Brisket	Hind leg
Municipal	B	1	+	+	-	+	+
	B	5	+	-	-	-	-
	C	3	+	-	-	-	-
	D	3	-	+	-	-	-
	D	4	+	-	-	-	+
	E	2	-	+	-	-	-
	E	5	+	-	-	-	-
	F	3	+	-	-	-	-
	F	4	-	-	-	-	+
	G	3	-	-	-	+	-
Private	A	2	+	+	-	-	-
	A	3	-	-	+	-	-
	C	4	+	-	-	+	-
	D	1	-	-	-	-	+
	D	2	+	-	-	-	-
	D	3	-	+	-	-	-
	D	4	-	+	-	-	-
	D	5	-	+	-	-	-
	E	1	+	-	-	-	-
	E	5	+	-	-	-	-
	F	2	-	-	-	+	-
	F	5	-	-	-	-	+
	G	3	-	+	+	-	-

 +Sample positive for *Salmonella*; + Sample positive for *E. coli* O157
 * identification number of each animal/visit

Gutema et al. (2021b) reported 7.1% positive rectal contents collected from cattle in the lairage at the same slaughterhouses. The 4.3% positive hides was lower compared to the global prevalence estimate of 44% with a variation from 7.3 to 76% (Rhoades et al. 2009). Only from one carcass, *E. coli* O157 was detected from the rectal content and the hide concomitantly. However, genetic typing showed that the isolates from both samples were not identical, indicating that the feces of the animal was not the source of the hide contamination.

In the present study, we observed a carcass contamination rate of 8.6 and 7.1% for *Salmonella* and *E. coli* O157, respectively. The carcass contamination with *Salmonella* was comparable to the prevalence of 7.6% (Muluneh and Kibret 2015), 12.5% (Wabeto et al. 2017)

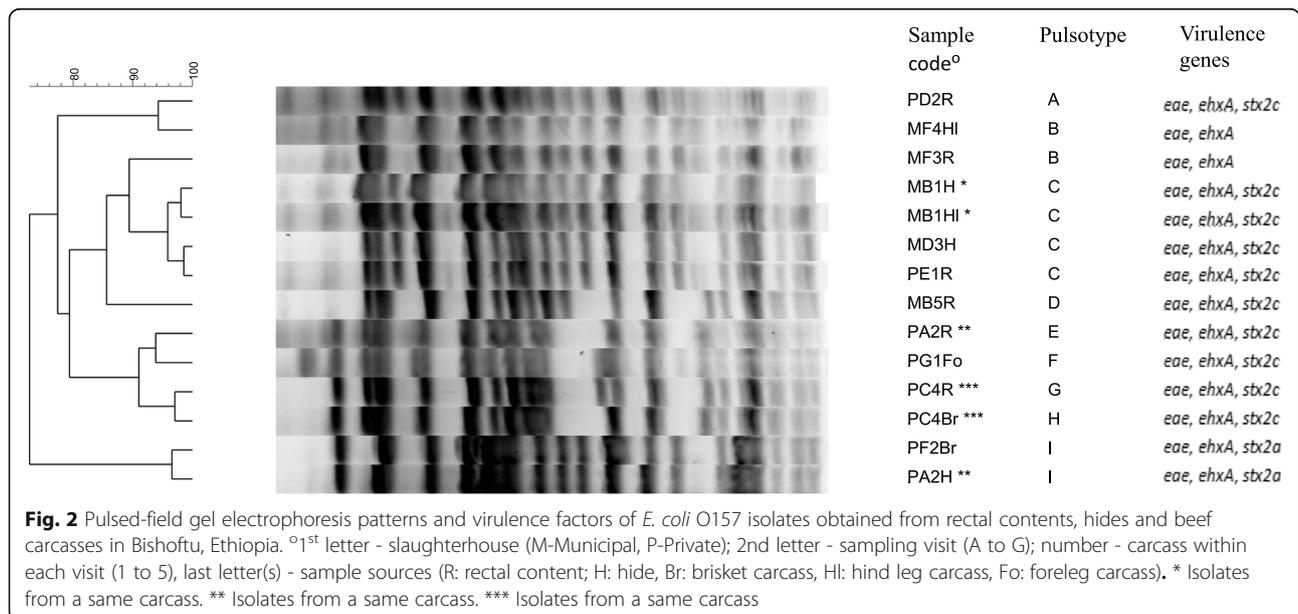
and 11.3% (Takele et al. 2018) in Ethiopia. For *E. coli* O157 variable proportions of carcass contamination were reported: from 0.54% by Abdissa et al. (2017) up to 13.3% by Bekele et al. (2014) for Ethiopia. For both pathogens, positive carcasses were only found positive on one carcass site, indicating that the carcass contamination is in most cases not widespread over the positive carcasses. As a consequence, collecting swab samples from multiple sites on a carcass may increase the number of positive carcasses. According to the EU regulation 2073/2005 beef carcasses sampled for bacteriological analysis, four carcass sites have to be swabbed (European Commission 2005). However, such regulation is not available for Ethiopia. Thus, it is essential to include adequate number of carcass sites to be sampled for



bacteriological analysis in the beef safety regulation of Ethiopia.

Only in a few cases *Salmonella* or *E. coli* O157 were detected simultaneously in the rectal content or on the hide, and on the corresponding carcasses. For the two *Salmonella* isolates and one of the three *E. coli* O157 isolates, respectively from the rectal content and hide, and the carcass swabs were genetically similar, indicating a possible direct transfer of the pathogen to the carcass

surface. In all other cases, no genetic link was stated between isolates from the rectal content and/or hide isolates and the carcass swabs. The observed low level of linkage of *Salmonella* and *E. coli* O157 isolates on carcass with those from rectal contents and/ or hide indicates that other sources may be involved in the carcass contamination during slaughter. This could be due to cross contamination caused by unhygienic handling practices such as infrequent washing of knives and hands



(Gutema et al. 2021a). Contamination and cross contamination of hides during cattle transport or in the lairage could increase the risk of carcass contamination.

Salmonella Dublin was isolated from the hide of one carcass and from the foreleg of another carcass in this study and it was also previously reported from retail beef in Ethiopia (Ejeta et al. 2004) indicating that this serotype is present in the cattle population and can be a source for human infections. *S. Dublin* is known to cause invasive infections and fatalities in humans (Harvey et al. 2017; Mattheus et al. 2018). In Ethiopia, *S. Dublin* was sporadically reported from stool of diarrheic patients (Tadesse 2014).

All *S. Typhimurium* isolates were multidrug resistant being resistant to ampicillin, chloramphenicol, sulfamethoxazole and tetracycline. Except for chloramphenicol, similar resistance profile was observed for *S. Typhimurium* isolates from cattle before slaughter at the same slaughterhouses (Gutema et al. 2021c). This suggests the widespread occurrence of ampicillin, sulfonamides and tetracycline resistance in *S. Typhimurium* isolated from cattle in Ethiopia and may be related to the long-time marketing and accessibility of these drugs. The two *S. Dublin* isolates were resistant to colistin. Resistance to this antibiotic seems to be common in *S. Dublin* isolates (EFSA and ECDC 2020). The finding that all 14 *E. coli* O157 isolates being susceptible to the 14 antimicrobials tested was in agreement with our previous study that have reported susceptibility of *E. coli* O157 isolates except one isolate obtained from beef in the study area (Gutema et al. 2021a).

Conclusions

During slaughter, beef carcasses can become contaminated with *Salmonella* and/or *E. coli* O157. The contamination was not widespread over positive carcasses. The study indicated the frequent occurrence of cross contamination besides the direct contamination of carcasses by feces and hide of positive animals. More studies are needed to unravel the sources for this cross contamination and to develop efficient preventive measures to ensure beef safety.

Abbreviations

BPW: Buffered Peptone Water; CT-SMAC: Cefixime Tellurite Sorbitol MacConkey Agar; ERIC: Enterobacterial Repetitive Intergenic Consensus; EUCAST: European Committee on Antimicrobial Susceptibility Testing; EUVSEC: Sensititre European Union Surveillance *Salmonella/E.coli*; FBD: Foodborne diseases; IMS: Immunomagnetic Separation; MIC: Minimum inhibitory concentration; MSRV: Modified Semi Solid Rappaport-Vassiliadis Medium; mTSBn: Modified Tryptone Soya Broth supplemented with novobiocin; PFGE: Pulsed Field Gel Electrophoresis; XLD: Xylose Lysine Deoxycholate; UPGMA: Unweighted-pair group method using arithmetic averages

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40550-021-00082-1>.

Additional file 1 : Supplementary Table 1. Distribution of the minimum inhibitory concentrations (MICs) of *Salmonella* isolates obtained from rectal contents ($n = 5$), hide swabs ($n = 5$) and carcasses ($n = 6$) in Bishoftu town, Ethiopia

Acknowledgments

We thank Tsedale Teshome, Sjarlotte Willems and Eline Dumoleijn for technical support. Mention of trade names or commercial products by USDA author (GEA) in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

Authors' contributions

All the authors made significant contributions to the manuscript and agree to its publication. The contributions were performed in the following order: Conceived and designed the study: FDG, RDA, GEA, LD, SG, LDZ. Performed laboratory analysis: FDG, SF, GR, FC, WM. Analyzed the data: FDG, LDZ, GR. Drafted the manuscript: FDG. Critically revised the manuscript: FDG, RDA, GEA, GR, SG, LDZ. All the authors read and approved the final manuscript.

Funding

This work was supported by Addis Ababa University and Ghent University under the Special Research Fund (BOF) program for developing countries (Scholarship's code 01 W03916).

Availability of data and materials

All data generated during this study are included in the manuscript.

Declarations

Competing interests

All the authors state that they have no competing interests in this study.

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Received: 10 March 2021 Accepted: 28 May 2021

Published online: 04 July 2021

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Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

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