

<https://helda.helsinki.fi>

Bacterial quality and safety of raw beef : A comparison between Finland and Nigeria

Osemwowa, Etinosa

2021-12

Osemwowa , E , Omoruyi , I M , Kurittu , P , Heikinheimo , A & Fredriksson-Ahomaa , M
2021 , ' Bacterial quality and safety of raw beef : A comparison between Finland and Nigeria
' , Food Microbiology , vol. 100 , 103860 . <https://doi.org/10.1016/j.fm.2021.103860>

<http://hdl.handle.net/10138/333937>

<https://doi.org/10.1016/j.fm.2021.103860>

cc_by

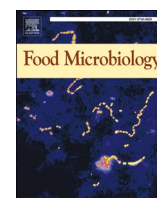
publishedVersion

Downloaded from Helda, University of Helsinki institutional repository.

This is an electronic reprint of the original article.

This reprint may differ from the original in pagination and typographic detail.

Please cite the original version.



Bacterial quality and safety of raw beef: A comparison between Finland and Nigeria

Etinosa Osemwowa^a, Iyekohtin Matthew Omoruyi^b, Paula Kurittu^a, Annamari Heikinheimo^a, Maria Fredriksson-Ahomaa^{a,*}

^a Department of Food Hygiene and Environmental Health, Faculty of Veterinary Medicine, University of Helsinki, Finland

^b Department of Biological Sciences, Faculty of Basic and Applied Sciences, Benson, Idsa University, Benin City, Nigeria

ARTICLE INFO

Keywords:

Beef
Bacterial contamination
Mesophilic aerobic bacteria (MAB)
Thermotolerant coliform bacteria (TCB)
Foodborne pathogens
Cephalosporin-resistant *Escherichia coli* (CREC)
Detection

ABSTRACT

Beef can easily be contaminated with bacteria during the meat production chain. In this work, we studied the contamination levels of mesophilic aerobic bacteria (MAB) and thermotolerant coliform bacteria (TCB) on raw beef surfaces from small shops in Helsinki, Finland and meat markets in Benin City, Nigeria. We also investigated the prevalence of *Salmonella*, *Campylobacter*, *Yersinia*, Shiga toxin-producing *Escherichia coli* (STEC), *Listeria*, and cephalosporin-resistant *E. coli* (CREC). In total, one hundred unpacked raw beef samples from Finland and Nigeria were collected in 2019. The median MAB and TCB counts were significantly ($P < 0.001$) higher on beef from Nigeria than from Finland. The median MAB and TCB counts in Nigeria were 7.5 and 4.0 \log_{10} cfu/cm², respectively, and 6.5 and 2.8 \log_{10} cfu/cm² in Finland, respectively. Most (94%) Nigerian samples were unacceptable according to limits set by the EU. Beef samples from meat markets in Benin City were significantly ($P < 0.05$) more frequently contaminated with *Salmonella*, STEC, and CREC than beef samples from small shops in Helsinki. *Salmonella*, STEC, and CREC were isolated from 30, 36, and 96% of Nigerian samples, respectively, and from <2, 12, and 2% of Finnish samples, respectively. Our study demonstrates a significant difference between the bacterial contaminations of raw beef in Nigeria and Finland, along with a possible misuse of cephalosporins in animal husbandry in Nigeria.

1. Introduction

Meat, including beef, is an important source of high-quality nutrition, such as proteins and essential amino acids, valuable vitamins and trace elements for humans (Hocquette et al., 2018). Meat consumption is highest in high-income countries, including Finland, and lowest in low-income countries (Godfray et al., 2018). In Africa, the demand for meat is increasing and meat consumption may be a status symbol, especially in low-income countries (Zerabruk et al., 2019). However, improper handling and sanitation practices along the meat production chain may lead to high bacterial contamination of the meat and meat contact surface (Rani et al., 2017). The rich protein content and sufficient water activity enable the easy growth of bacteria in the meat, leading to spoilage. Spoiled meat is unpleasant and unsuitable for consumption, causing enormous economic loss (Azuamah et al., 2018). Meat production and consumption are also associated with potential food safety risks (Li et al., 2019).

Foodborne infections are common worldwide, exhibiting clear regional variations (Hoffmann et al., 2017). Foodborne infections related to meat are a global public health concern due to the high risk of bacterial contamination of meat by several types of pathogens (Tesson et al., 2020). Non-typhoidal *Salmonella enterica*, *Campylobacter*, and Shigatoxin-producing *Escherichia coli* (STEC) are common findings worldwide in livestock including cattle, which is an important reservoir of these pathogens and an important contamination source of raw beef (Li et al., 2019). *Campylobacter*, *Salmonella*, STEC, and enteropathogenic *Yersinia* are the most common bacterial pathogens causing foodborne diseases in Europe, and *Salmonella* and STEC are the most important public health hazards associated with contaminated raw beef (EFSA and ECDC, 2019). *Listeria monocytogenes* can be found in many foods in the EU, including meat. Listeriosis is a relatively rare disease, but it is one of the most severe foodborne diseases, with a high mortality rate especially among the elderly and immunosuppressive patients (Chlebicz and Śliżewska). Beef is also a suspected exposure route for

* Corresponding author.

E-mail address: maria.fredriksson-ahomaa@helsinki.fi (M. Fredriksson-Ahomaa).

antimicrobial-resistant bacteria entering humans (Zhang et al., 2020). Gram-negative bacteria in particular, which are resistant to critically important antimicrobials (e.g. 3rd generation cephalosporines and quinolones) used in human medicine, have become an increasing problem (World Health Organization, 2017).

Consumer demand for beef is increasing in Nigeria (Famubo et al., 2020). However, hygiene practices remain poor along the meat production chain, and one of our aims was therefore to study bacterial contamination on the surface of raw beef at the retail level in Nigeria. Furthermore, we wanted to compare the bacterial contamination levels and presence of foodborne pathogens on raw beef surfaces from open meat markets in Benin City, Nigeria with raw beef from small-scale shops in Helsinki, Finland. Small-scale meat shops, including halal meat shops, are rare in Finland and have not been studied before. We also wanted to investigate the prevalence of 3rd generation cephalosporin -resistant *E. coli* on beef in Nigeria and Finland, which are two countries with differing usage levels and control of antimicrobials.

2. Materials and methods

2.1. Sampling and sample preparation

In total, 100 unpacked raw beef samples from Finland and Nigeria were studied in 2019 (Table 1). Meat cuts were bought from five (A–E) small-scale halal meat shops in Helsinki, Finland on eight days and from five open meat markets (F–I) located in Benin City, Nigeria on five different days. A sterile cotton bud was used to swab a 10 cm × 10 cm surface area of the meat cut. Meat samples included five meat types (Table 1). Only one cut of the same meat type was collected from same vendor per day. In total, 11 samples from the brisket, 9 from the round, and 10 each from the chuck, fore and hindshank were sampled in both countries.

2.2. Bacterial counts

Each swab was mixed in 10 ml of buffered peptone water for 60 s (BPW, Labema, Kerava, Finland). The number of mesophilic aerobic bacteria (MAB) and thermotolerant coliform bacteria (TCB), also called presumptive *Escherichia coli*, were determined using Nordic guidelines: NMKL86 for MAB and NMKL125 for TCB (NMKL, 2005, 2013). Tenfold dilutions (10^{-1} – 10^{-8}) were plated in duplicate on plate count agar (PCA, VWR, Germany) and violet red bile (VRB, Labema) agar plates for MAB and TCB, respectively. PCA plates were incubated at 30 °C for 24–48 h and TCB plates at 44 °C for 24 h.

Table 1

Beef samples collected in Helsinki, Finland (N = 50) and in Benin City, Nigeria (N = 50) in 2019.

Country	Sampling location ^a	Number of samples ^b	Meat type				
			Brisket	Foreshank	Hindshank	Round	Chuck
Finland	A	16	2	4	4	2	4
	B	9	0	3	3	0	3
	C	8	0	3	2	0	3
	D	9	5	0	1	3	0
	E	8	4	0	0	4	0
Nigeria	F	10	2	2	1	3	2
	G	10	0	3	3	0	4
	H	10	3	2	2	2	1
	I	10	2	1	2	3	2
	J	10	4	2	2	1	1

^a Small-scale halal meat shops in Helsinki and open meat markets in Benin City.

^b In Finland, 2–3 meat types from 1 to 3 shops per day over 8 days were collected and in Nigeria, 2–3 meat types from 2 markets over 5 days were collected.

2.3. Pathogen screening by PCR

Presences of the *rrn* gene from *Campylobacter*, *ttr* from *Salmonella*, *ail* from *Yersinia*, *stx* from *E. coli*, and *mpl* from *Listeria* were screened by PCR according to Sauvala et al. (2019). DNA was extracted from 100 µl of the overnight enrichment (ON for 18–20 h at 37 °C BPW) using Chelex™100 resin (BioRad, Hercules, California). PCR-positive samples were plated on selective agar plates (Labema) after ON: an XLD plate for *Salmonella*, CHROMagar™STEC for STEC, and CHROMagar™*Listeria* for *Listeria* (Labema). All plates were incubated at 37 °C for 24–48 h. *Salmonella* and STEC isolates were identified by PCR targeting *ttr* for *Salmonella* and *stx1* and *stx2* for STEC. Additionally, the isolates were identified with an API20E test (Biomérieux, Marcy-l'Étoile, France). Serotyping of *L. monocytogenes* was performed by commercial antisera (Denka Seiken, Japan).

2.4. Isolation of 3rd generation cephalosporin -resistant *Escherichia coli* (CREC)

From the ON enrichment, 100 µl was plated on MacConkey agar (Labema) supplemented with 1 mg/l cefotaxime and incubated at 44 °C for 18–24 h. Typical colonies were plated on blood agar for a pure culture. Identification was performed by gram staining, an oxidase test, and an API20E test (Biomérieux). Antimicrobial susceptibility testing was conducted using the disc diffusion method for two 3rd generation cephalosporins: cefotaxime 5 µg (Oxoid, Basingstoke, UK) and ceftazidime 10 µg (Neo-Sensitabs, Rosco, Denmark). An isolate was resistant to 3rd generation cephalosporins if it showed zone sizes for cefotaxime (5 µg) below 21 mm and below 22 mm for ceftazidime (10 µg) according to EUCAST (Giske et al., 2018).

2.5. Antimicrobial susceptibility testing with a broth microdilution method

Antimicrobial susceptibility was tested according to CLSI guidelines (Clinical Laboratory standards, 2018) using a broth microdilution method (VetMIC™, National Veterinary Institute SVA, Uppsala, Sweden). The panel of 15 antimicrobial agents included in the VetMIC GN-mo (version 9) was used for all *Salmonella* isolates and for 6 CREC isolates including the only isolate found in Finland and 5 from different markets in Nigeria.

2.6. Statistical analyses

SPSS®26 statistical software (IBM Corp., Armonk, NY, USA) was used for statistical analyses. MAB and TCB counts were log-transformed before analyses. Normality of continuous variables (MAB and TCB) was tested with the Shapiro-Wilk test. Non-parametric tests were used for

both continuous (MAB and TCB) and categorical (country, pathogen) variables. Mann-Whitney *U* test and Kruskal-Wallis test were used for continuous variables and Fisher's exact test for categorical variables. $P < 0.05$ was statistically significant.

3. Results

The median values of MAB on raw beef surfaces from Finland and Nigeria were 6.7 and 7.5 \log_{10} cfu/cm², respectively, and the median TCB values were 2.8 and 4.0 \log_{10} cfu/cm², respectively (Table 2). The median values of both MAB and TCB were significantly higher on beef from Nigeria than from Finland (Mann-Whitney *U* test, $P < 0.001$).

Contamination levels between different meat sample types from Finland differed significantly when adjusted by Bonferroni correction (Kruskal-Wallis test, $P < 0.01$): the highest median MAB count of 6.9 \log_{10} cfu/cm² was found on beef samples originating from the brisket and the lowest (5.7 \log_{10} cfu/cm²) were found on the hindshank meat. The highest median TCB counts (3.0 and 3.1 \log_{10} cfu/cm²) were observed on the brisket and round meats, respectively, and the lowest (2.6 \log_{10} cfu/cm²) were found from the shank samples. No significant differences ($P > 0.05$) were obtained between the sample types from Nigeria. Significant differences (Kruskal-Wallis test, $P < 0.02$) were also observed between the shops in Finland but not between the markets in Nigeria. The median MAB and TCB counts were highest in samples from shops D and E. In these shops, almost all samples (16/17) were taken from the brisket and round meats (Table 1). We found some significant differences between the median MAB and TCB counts for different sampling days on beef surface samples from Finland. The highest median counts of MAB and TCB were observed on two out of eight sampling days. On these days, most samples (16/20) were collected from shops D and E.

Almost 50% of the raw beef samples from small-scale shops in Finland were unsatisfactory based on limits for MAB counts set by the EU for raw beef (Table 3). However, all samples were at least acceptable based on the TCB counts. Our limits for TCB were based on the limits for *E. coli* set by the EU and multiplied by 10 (Table 3). Nearly all (94%) raw beef samples from open meat markets in Nigeria were unsatisfactory based on the MAB counts and all were unsatisfactory based on the TCB counts (Table 3).

Salmonella (*ttr*) and STEC (*stx*) were frequently detected on the

Table 2

Median values (\log_{10} cfu/cm²) of mesophilic aerobic bacteria (MAB) and thermotolerant coliform bacteria (TCB) found on the surface of raw beef in Finland and Nigeria in 2019.

Country	Sampling	Number of samples	MAB		TCB	
			Median	Min-Max	Median	Min-Max
Finland		50	6.7	4.3–8.0	2.8	1.8–3.4
Meat type	Brisket	11	6.9	6.4–8.0	3.0	2.0–3.4
	Chuck	10	6.5	4.5–7.4	2.7	1.8–3.1
	Froreshank	10	6.3	4.8–6.9	2.6	2.5–3.0
	Hindshank	10	5.7	4.3–6.9	2.6	1.8–3.0
	Round	9	6.7	6.5–7.8	3.1	2.7–3.2
Shop	A	16	6.3	4.3–7.8	2.7	2.2–3.1
	B	9	6.4	4.8–7.4	2.7	2.5–3.0
	C	8	6.4	5.3–7.0	2.6	1.8–3.0
	D	9	6.9	6.5–8.0	3.0	2.7–3.2
	E	8	6.9	6.5–7.4	3.0	2.0–3.4
Nigeria		50	7.5	6.6–9.4	4.0	3.8–4.2
Meat type	Brisket	11	7.5	6.6–8.2	4.0	3.8–4.2
	Chuck	10	7.8	7.2–9.4	4.0	3.9–4.2
	Foreshank	10	7.8	7.1–8.2	3.9	3.8–4.1
	Hindshank	10	7.6	7.1–8.9	3.9	3.8–4.2
	Round	9	7.5	6.6–8.7	4.1	3.9–4.2
Meat market	F	10	7.1	6.6–8.0	4.0	3.8–4.2
	G	10	7.9	7.1–8.1	4.0	3.8–4.2
	H	10	7.4	7.1–8.7	4.0	3.8–4.1
	I	10	7.5	7.3–7.8	4.0	3.9–4.1
	J	10	7.8	6.8–9.4	4.0	3.8–4.2

Table 3

Mesophilic aerobic bacteria (MAB) and thermotolerant coliform bacteria (TCB) counts found on beef in Finland and Nigeria.

Bacteria	Limits ^a	Log cfu ₁₀ /cm ²	Judgement	No. of carcasses	
				Finland N = 50	Nigeria N = 50
MAB	m	<5.7	Satisfactory	12 (24%)	0
		5.7–6.7	Acceptable	15 (30%)	3 (6%)
TCB	M	>6.7	Unsatisfactory	23 (46%)	47 (94%)
		<2.7	Satisfactory	19 (38%)	0
	m ^b	2.7–3.7	Acceptable	31 (62%)	0
		>3.7	Unsatisfactory	0	50 (100%)

^a Limits for raw meat in Commission Regulation (EC) No 2073/2005.

^b *Escherichia coli* limits were multiplied by 10.

surface of raw beef samples in Nigeria by PCR (Table 4). No *Salmonella* was detected on beef samples in Finland. Both *Salmonella* (*ttr*) and STEC (*stx*) were significantly (Fisher's exact test, $P < 0.001$) more often detected on beef samples in Nigeria. *Campylobacter* (*rrn*) was detected on 2% of the samples and no *Yersinia* (*ail*) was detected in either country. *Listeria* (*mpl*) was detected more often on raw beef in Finland than in Nigeria; however, the difference was not significant ($P > 0.05$).

Salmonella spp. was isolated from 15 of 21 PCR-positive raw beef samples from meat markets in Nigeria but not from any shops in Finland where it was not detected by PCR (Table 5). STEC was isolated from 18 of 50 PCR-positive beef samples from Nigerian meat markets. Most (17/18) of the culture-positive samples were contaminated with *stx2*-positive STEC. STEC was isolated from six of 9 PCR-positive beef samples in Finland, four of which were *stx1*-positive and two were *stx2*-positive STEC. Most (96%) beef samples from Nigerian meat markets were contaminated with CREC, while only one (2%) sample in Finland was contaminated. *L. monocytogenes* was isolated from only one raw beef sample, which originated from a meat shop in Finland. It belonged to serotype 1/2a. No *Campylobacter* was isolated from any beef samples.

Salmonella isolates from 15 Nigerian beef samples were sensitive to most (14/15) of the tested antimicrobials (Table 6). Only resistance to ciprofloxacin was observed. Additionally, we also tested six CREC isolates (one from Finland and 5 from Nigeria) with the VetMIC™ test. All

Table 4
Prevalence of foodborne pathogens on raw beef surfaces in Finland and Nigeria by PCR.

Pathogen	Target gene	Finland (N = 50)			Nigeria (N = 50)		
		Positives	%	95%CI ^a	Positives	%	95%
<i>Campylobacter</i>	<i>rrn</i>	1	2.0	0.1–10.6	1	2.0	0.1–10.6
<i>Salmonella</i>	<i>ttr</i>	0 ^b	<2.0	0.0–7.1	21 ^c	42.0	28.2–56.8
<i>Yersinia</i>	<i>ail</i>	0	<2.0	0.0–7.1	0	<2.0	0.0–7.1
STEC	<i>stx1+stx2</i>	9 ^b	18.0	8.6–31.4	50 ^c	100.0	92.9–100.0
<i>Listeria</i>	<i>mpl</i>	16	32.0	19.5–46.7	9	18.0	8.6–31.4

^a Clopper-Pearson exact test.

^b And ^c differ significantly from each other at the 0.05 level using Fisher's exact test with Bonferroni correction.

Table 5
Isolation rates of *Salmonella*, *Listeria monocytogenes*, *stx*-positive *Escherichia coli* (STEC), and 3rd generation cephalosporin -resistant *Escherichia coli* (CREC) on raw beef meat.

Bacterium	Number of positive samples	
	Finland (N = 50)	Nigeria (N = 50)
<i>Salmonella</i>	0 (<2%)	15 (30%)
<i>Listeria monocytogenes</i>	1 (2%)	0 (<2%)
STEC	6 (12%)	18 (36%)
CREC	1 (2%)	48 (96%)

isolates were resistant to β -lactams and quinolones (Table 6). Multi-resistance was observed in five out of six isolates.

4. Discussion

We assessed the bacterial quality of raw beef at the retail level in Finland and Nigeria with MAB and TCB counts. We were able to demonstrate that the bacterial load on the raw beef surfaces from meat markets in Benin City were significantly higher compared with the raw beef from small-scale shops in Helsinki. However, the bacterial load of the Nigerian beef was slightly lower compared with the bacterial load of raw beef recently reported in Ethiopia (Teshome et al., 2020). Only 38% of the Nigerian samples could be judged as satisfactory according to EU limits for MAB (Commission regulation (EC) 2073/2005). For the TCB counts, we used the EU limits for *E. coli* multiplied by 10. Using these limits, all the beef sampled in Nigeria were unsatisfactory. No Nigerian microbial limits for raw meat are available. The high bacterial load of

beef meat is most probably due to poor hygiene and sanitation during slaughtering, transportation, and handling at the retail level (Teshome et al., 2020). Also, storage at abusive temperatures allows bacterial growth. The very high TCB counts in Nigeria indicate high contamination during the meat production chain combined with improper storage temperatures.

Almost 50% of the raw beef samples from small-scale shops in Finland were unsatisfactory according to the MAB counts, which indicates low microbial quality and inappropriate shelf lives of these products. The beef samples were collected from small shops where meat was sold unpackaged. Poor hygiene during handling may be one reason for the relatively high bacterial loads. However, no beef samples were unsatisfactory according to the TCB counts, showing relatively low *E. coli* contamination in Finland.

The beef samples included five meat types (brisket, chuck, fore/hind shanks, and round meat) collected from five meat markets in Nigeria, and no significant differences in bacterial counts were observed between the sample types and sampling locations. A significantly higher microbial load was observed in Finland on the surface of the brisket and round meats, and on the samples from shops D and E. Interestingly, nearly all samples collected from shops D and E were brisket and round meats. Whether the meat type was the reason for the higher bacterial loads in shops D and E or whether the hygiene practices were improper in these shops remains unclear. The bacterial counts were highest on two sampling days, and most of the samples on these days were from shops D and E, which further indicates that these shops had hygiene problems. No significant differences were observed between the sampling days in Nigeria.

Very high prevalence of *Salmonella* (42%) and STEC (100%) were

Table 6
Antimicrobial susceptibility among *Salmonella* and 3rd generation cephalosporin-resistant *Escherichia coli* (CREC) isolates using the VetMIC™ test.

Antimicrobial Agent	Antimicrobial classes	<i>Salmonella</i> (N = 15)		CREC (N = 6 ^b)	
		MIC ^a (mg/ml)	Susceptible isolates	MIC (mg/ml)	Susceptible isolates
Ampicillin	B-Lactam	8	15	8	0
Cefotaxime	B-Lactam	0.5	15	0.25	0
Ceftazidime	B-Lactam	2	15	0.5	0
Streptomycin	Aminoglycoside	16	15	16	1
Gentamycin	Aminoglycoside	2	15	2	5
Chloramphenicol	Chloramphenicol	16	15	16	4
Florfenicol	Chloramphenicol	16	15	16	6
Ciprofloxacin	Quinolone	0.06	7	0.06	0
Enrofloxacin	Quinolone	0.25	15	0.125	0
Nalidixic acid	Quinolone	8	15	8	5
Tetracycline	Tetracycline	8	15	8	2
Sulfamethoxazol	Sulphonamide	256	15	64	2
Trimethoprim	Diaminopyrimidine nhibitor (DI)	2	15	2	2
Trimethoprim/Sulfamethoxazole	DI/Sulphonamide	0.5	15	0.25	3
Colistin	Polymyxin	2	15	2	6

^a Minimum inhibitory concentration using epidemiological cut-off values (ECOFF) according to EUCAST 2021 (<https://mic.eucast.org/search/>).

^b CREC isolates including five from Nigeria and the only one found in Finland.

found on the Nigerian raw beef samples by PCR screening. The isolation rates of *Salmonella* (30%) and STEC (36%) were also high, which suggests high contamination levels of these pathogens in the Nigerian samples. *Salmonella* prevalence on Nigerian beef surfaces in our study was higher compared to studies reported in a recent review from Africa (Thomas, K. M. et al., 2020). However, a similar isolation rate (30%) of STEC in beef products in Benin City, Nigeria has been reported before (Omoruyi et al., 2018). No *Salmonella* was detected in Finland, and the STEC prevalence was also significantly lower than in Nigeria. One explanation for the lack of *Salmonella* detected in Finland is most probably the very low (<1%) *Salmonella* prevalence in beef due to the highly effective Finnish *Salmonella* Control Program (Maijala et al., 2005). The significantly lower TCB counts on Finnish beef surface samples compared with Nigerian samples most likely also reflects the significantly lower STEC counts detected on Finnish samples. A recent review on the quantitative risk assessment of beef meat reported that carrier animals at the farm, dehiding, and chilling at the slaughterhouse, along with storage temperatures at the retail level were the most important risk factors for *Salmonella* and STEC contamination in beef meat (Tesson et al., 2020).

No enteropathogenic *Yersinia* were detected on any beef meat surface samples. Pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* have rarely been detected in cattle and beef, which may explain the negative results for beef in our study (Fredriksson-Ahomaa et al., 2018). However, enteropathogenic *Yersinia* have sporadically been found in pigs and sheep in Nigeria in an earlier study (Okwori et al., 2009). *Campylobacter* was detected very rarely (2%) in both countries by PCR. Beef surface, especially when dry, does not foster the growth or even the survival of *Campylobacter* (Murphy et al., 2006). The risk of foodborne transmission of *Campylobacter* through beef has recently been shown to be low (Inglis et al., 2020). *Listeria* prevalence was higher on beef surfaces in Finland (32%) compared with Nigeria (18%) by PCR. One reason can be that the samples in Finland were refrigerated but not in Nigeria. *L. monocytogenes* was isolated from only one sample in Finland. It belonged to serotype 1/2a, which is one of the most common serotypes associated with human listeriosis (Chlebicz and Śliżewska). *L. monocytogenes* may cause public health problems, especially if the contaminated beef is stored at refrigerator temperatures for a prolonged time, which favors the growth of psychotropic bacteria, such as *Listeria*. A listeriosis outbreak was recently reported in South Africa, which was linked to contaminated ready-to-eat processed meat containing beef (Thomas, J. et al., 2020).

The prevalence of 3rd generation cephalosporin-resistant *E. coli* on the beef meat surfaces was very high in Nigeria. CREC was isolated from almost all (96%) Nigerian meat samples but from only one Finnish sample. One reason for the high prevalence of CREC was probably the very high TCB counts on the meat surfaces, indicating fecal contamination of the meat samples in Nigeria. Another reason may be common overuse and misuse of antimicrobials in husbandry in Africa, which may lead to increased numbers of antimicrobial-resistant bacteria (Alonso et al., 2017). In Nigeria, farmers can purchase veterinary drugs without veterinary prescriptions or supervision (Adesokan et al., 2015; Alhaji and Isola, 2018). The low prevalence of CREC (2%) on the beef surface samples in Finland may partly be explained by the strict control and documented use of antimicrobials in husbandry and because cephalosporins are very rarely used for treating diseases in food animals in Finland (Nykänen et al., 2019). We also studied selected CREC isolates with the broth microdilution method. All isolates were resistant to β -lactams, including 3rd generation cephalosporins and quinolones, but resistance to several other antimicrobials was also shown.

Multiresistant *Salmonella* has previously been found in a beef slaughterhouse in Nigeria from the environment, equipment, and beef carcasses (Okafor et al., 2020). Surprisingly, all *Salmonella* isolates in our study were sensitive to most (14/15) of the antimicrobials, including cephalosporins, when using the broth microdilution method. Susceptibility to 3rd generation cephalosporins was confirmed with the disc

diffusion method. Only resistance to ciprofloxacin (53%) was observed. Ciprofloxacin resistance among *Salmonella* isolates from food animals in Nigeria has been confirmed in a review (Oloso et al., 2018). Tetracyclines, β -lactams/aminoglycosides, sulfonamides, and fluoroquinolones have been reported to constitute the majority of antimicrobials used in husbandry in Nigeria (Adesokan et al., 2015; Alhaji and Isola, 2018). Increasing prevalence of cephalosporin resistance and plasmid-mediated quinolone resistance genes have been reported in Africa (Alonso et al., 2017). Overuse and misuse of quinolones in Nigeria may be one reason for the quinolone resistance observed among *Salmonella* and CREC isolates in our study.

5. Conclusions

Bacterial contamination levels of raw beef meat were significantly higher in Nigeria than in Finland, indicating poor working hygiene and sanitation along the meat production chain in Nigeria. The TCB count and the prevalence of *Salmonella*, STEC, and CREC were also high on the meat surfaces from Nigeria, indicating heavy contamination and likely improper cold storage. Proper heat treatment of beef meat is essential to avoid public health risks, especially in Nigeria. A high prevalence of CREC was observed on Nigerian beef meat surfaces, indicating the high use of cephalosporins in husbandry.

Funding

This work was partly supported by a travel grant from the Doctoral Program in Food Chain and Health, University of Helsinki.

Authors' contributions

EO performed the sampling and microbiological studies in Finland, EO and MO performed the sampling and microbiological studies in Nigeria. PK and AH performed the resistance studies and MFA the PCR and statistical studies. EO and MFA wrote the manuscript, which was edited by all authors. The final version was approved by all authors.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

This research was supported by a travel grant from the Doctoral School in Environmental, Food and Biological Sciences (YEB), University of Helsinki. Maria Stark and Kirsi Ristkari are gratefully acknowledged for their laboratory assistance.

References

- Adesokan, H.K., Akanbi, I.O., Akanbi, I.M., Obaweda, R.A., 2015. Pattern of antimicrobial usage in livestock animals in south-western Nigeria: the need for alternative plans. *Onderstepoort J. Vet. Res.* 82, 1.
- Alhaji, N.B., Isola, T.O., 2018. Antimicrobial usage by pastoralists in food animals in North-central Nigeria: the associated socio-cultural drivers for antimicrobials misuse and public health implications. *One Health* 6, 41–47.
- Alonso, C.A., Zarazaga, M., Ben Sallem, R., Jouini, A., Ben Slama, K., Torres, C., 2017. Antibiotic resistance in *Escherichia coli* in husbandry animals: the African perspective. *Lett. Appl. Microbiol.* 64, 318–334.
- Azuamah, Y.C., Amadi, A.N., Iro, O.K., 2018. Bacteriological qualities of red meat (beef) and meat hygiene practices among meat handlers in Aba Metropolis, Nigeria. *Int. J. Health Sci. Res.* 8, 41–49.
- Chlebicz, A., Śliżewska, K., 2018. Campylobacteriosis, salmonellosis, yersiniosis, and listeriosis as zoonotic foodborne diseases: a review. *Int. J. Environ. Res. Publ. Health* 15, 863.
- EFSA and ECDC, 2019. The European Union one health 2018 zoonoses report. *EFSA Journal* 17, 5926.
- Famubo, J.A., Isiak, A., Abbas, Y.B., 2020. Bacteriological analysis of beef production chain in Birnin Kebbi metropolis of Kebbi State, Nigeria. *Journal of Advances in Microbiology* 64–76.

- Fredriksson-Ahooma, M., Joutsen, S., Laukkanen-Ninios, R., 2018. Identification of *Yersinia* at the species and subspecies levels is challenging. *Current Clinical Microbiology Reports* 5, 135–142.
- Giske, C.G., Martinez-Martinez, L., Cantón, R., Stefani, S., 2018. EUCAST Guidelines for Detection of Resistance Mechanisms and Specific Resistances of Clinical And/or Epidemiological Importance. EUCAST. Version 2.0: 2017.
- Godfray, H.C.J., Aveyard, P., Garnett, T., Hall, J.W., Key, T.J., Lorimer, J., Pierrehumbert, R.T., Scarborough, P., Springmann, M., Jebb, S.A., 2018. Meat consumption, health, and the environment. *Science* 361.
- Hocquette, J., Ellies Oury, M., Lherm, M., Pineau, C., Deblitz, C., Farmer, L., 2018. Current situation and future prospects for beef production in Europe—a review. *Asian-Australas. J. Anim. Sci.* 31, 1017.
- Hoffmann, S., Devleeschauwer, B., Aspinall, W., Cooke, R., Corrigan, T., Havelaar, A., Angulo, F., Gibb, H., Kirk, M., Lake, R., 2017. Attribution of global foodborne disease to specific foods: findings from a World Health Organization structured expert elicitation. *PLoS One* 12, e0183641.
- Inglis, G.D., Gusse, J.F., House, K.E., Shelton, T.G., Taboada, E.N., 2020. Clinically relevant *Campylobacter jejuni* subtypes are readily found and transmitted within the cattle production continuum but present a limited foodborne risk. *Appl. Environ. Microbiol.* 86.
- Li, M., Havelaar, A.H., Hoffmann, S., Hald, T., Kirk, M.D., Torgerson, P.R., Devleeschauwer, B., 2019. Global disease burden of pathogens in animal source foods. *PLoS One* 14, e0216545.
- Murphy, C., Carroll, C., Jordan, K.N., 2006. Environmental Survival Mechanisms of the NMKL, 2005. Thermotolerant coliform bacteria and *Escherichia coli*. Enumeration in food and feed. *NMKL* 12, 4.
- NMKL, 2013. Aerobic microorganisms. Determination in foods by the colony count method. *NMKL* 86, 5.
- Nykänenoja, S., Olkkola, S., Pohjanvirta, T., Biström, M., Kaartinen, L., Helin-Soilevaara, H., Aarnio, M., Raunio-Saarnisto, M., Kivilahti-Mäntylä, K., Nevalainen, M., 2019. FINRES-vet 2018: Finnish Veterinary Antimicrobial Resistance Monitoring and Consumption of Antimicrobial Agents. <https://helda.helsinki.fi/handle/10138/307519>.
- Okafor, U.C., Okafor, S.C., Ogugua, A.J., 2020. Occurrence of multidrug-resistant *Salmonella* in cattle carcass and contact surfaces in Kwata slaughterhouse, Awka, Anambra State, Nigeria. *International Journal of One Health* 6, 49–55.
- Okwori, A.E.J., Martínez, P.O., Fredriksson-Ahooma, M., Agina, S.E., Korkeala, H., 2009. Pathogenic *Yersinia enterocolitica* 2/O:9 and *Yersinia pseudotuberculosis* 1/O:1 strains isolated from human and non-human sources in the Plateau State of Nigeria. *Food Microbiol.* 26, 872–875.
- Oloso, N.O., Fagbo, S., Garbati, M., Olonitola, S.O., Awosanya, E.J., Aworh, M.K., Adamu, H., Odetokun, I.A., Fasina, F.O., 2018. Antimicrobial resistance in food animals and the environment in Nigeria: a review. *Int. J. Environ. Res. Publ. Health* 15, 1284.
- Omoruyi, I.M., Uwadiae, E., Mulade, G., Omoruku, E., 2018. Shiga toxin producing strains of *Escherichia coli* (STEC) associated with beef products and its potential pathogenic effect. *Microbiology Research Journal International* 1–7.
- Rani, Z.T., Hugo, A., Hugo, C.J., Vimiso, P., Muchenje, V., 2017. Effect of post-slaughter handling during distribution on microbiological quality and safety of meat in the formal and informal sectors of South Africa: a review. *S. Afr. J. Anim. Sci.* 47, 255–267.
- Sauvala, M., Laaksonen, S., Laukkanen-Ninios, R., Jalava, K., Stephan, R., Fredriksson-Ahooma, M., 2019. Microbial contamination of moose (*Alces alces*) and white-tailed deer (*Odocoileus virginianus*) carcasses harvested by hunters. *Food Microbiol.* 78, 82–88.
- Teshome, G., Assefa, Z., Keba, A., 2020. Assessment of microbial quality status of raw beef around Addis Ababa city, Ethiopia. *Afr. J. Food Sci.* 14, 209–214.
- Tesson, V., Federighi, M., Cummins, E., de Oliveira Mota, J., Guillou, S., Boué, G., 2020. A systematic review of beef meat quantitative microbial risk assessment models. *Int. J. Environ. Res. Publ. Health* 17, 688.
- Thomas, J., Govender, N., McCarthy, K.M., Erasmus, L.K., Doyle, T.J., Allam, M., Ismail, A., Ramalwa, N., Sekwadi, P., Ntshoe, G., 2020. Outbreak of listeriosis in South Africa associated with processed meat. *N. Engl. J. Med.* 382, 632–643.
- Thomas, K.M., de Glanville, W.A., Barker, G.C., Benschop, J., Buza, J.J., Cleaveland, S., Davis, M.A., French, N.P., Mmbaga, B.T., Prinsen, G., 2020. Prevalence of *Campylobacter* and *Salmonella* in African food animals and meat: a systematic review and meta-analysis. *Int. J. Food Microbiol.* 315, 108382.
- World Health Organization, 2017. Critically Important Antimicrobials for Human Medicine: Ranking of Antimicrobial Agents for Risk Management of Antimicrobial Resistance Due to Non-human Use. <https://apps.who.int/iris/bitstream/handle/10665/255027/9789241512220-eng.pdf>.
- Zerabruk, K., Retta, N., Muleta, D., Tefera, A.T., 2019. Assessment of microbiological safety and quality of minced meat and meat contact surfaces in selected butcher shops of Addis Ababa, Ethiopia. *J. Food Qual.* 2019.
- Zhang, Y., Schmidt, J.W., Arthur, T.M., Wheeler, T.L., Wang, B., 2020. A comparative quantitative assessment of human exposure to various antimicrobial-resistant bacteria among US ground beef consumers. *J. Food Protect.*