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African Journal of Microbiology Research

Full Length Research Paper

# Detection of microbial surface contamination and antibiotic resistant *Escherichia coli* on beef carcasses in Arusha, Tanzania

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Food serves as an important fomite for transmission of disease-causing and antibiotic resistant bacteria to humans. Because this may be an especially challenging problem in low-income countries, the level of microbial surface contamination and abundance of antibiotic resistant *Escherichia coli* on beef carcasses were estimated. Out of 125 surface swab samples (plated on MacConkey agar), 70 to 100% were positive to Gram-negative bacteria and *E. coli*, respectively. More than 50% of individual carcasses had bacterial loads below the maximum threshold recommended by the FAO. For carcasses in small- and medium-scale facilities, the average load of bacteria ranged between 0.8 and 1.5 log cfu/cm<sup>2</sup>, while carcasses in the large slaughter facility had an average loads of between 1.77 and 1.42 log cfu/cm<sup>2</sup>. Of the 1,272 *E. coli* isolates tested, 49.4% were resistant to at least one antibiotic. Isolates were frequently resistant to tetracycline (21.7%) and ampicillin (19.2%) while the frequency of resistance to the remaining nine antibiotics was <3%. In addition, 5.3% of isolates were multidrug resistant with 18 different phenotypes. The combination of resistance to ampicillin and tetracycline was the most common. Although, poor sanitation practices were observed, results reflect lower bacterial counts and limited prevalence of antibiotic resistant *E. coli* relative to other reports in the literature.

Key words: Escherichia coli, antibiotic resistance, slaughterhouse hygiene, meat contamination, public health.

# INTRODUCTION

There is a clear correlation between the use of unhygienic slaughterhouse practices and the incidence of meat-borne disease outbreaks (Sousa, 2008; Ali et al., 2010) in part, because the nutrient composition and water activity of meat is attractive to a broad spectrum of microorganisms (De Filippis et al., 2013). High microbial surface contamination of beef carcasses constitutes a significant risk to meat handlers and consumers

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**Table 1.** The acceptable level of bacterial load in red meat (USDA – FAO, 2013). All values are log-transformed colony forming units per square centimeter of surface sampled. Samples were collected after carcass is dressed.

Organisms	Acceptable	Critical/Marginal	Not acceptable
Total bacteria	<3.5	3.5-5.0	> 5.0
Total coliforms	<1.5	1.5-2.5	> 2.5
E. coli	≤ 0.8	> 0.8 and ≤ 1.8	> 1.8

especially during processing and/or eating contaminated meat or meat products (Heiman et al., 2015). In an effort to combat these challenges, the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) have established maximum microbiological surface contamination guidelines for bacterial load on the surface of meat carcasses (Table 1). In many instances, carcass surfaces have been contaminated with enteric bacteria during evisceration and/or from the contaminated tools and surfaces of slaughterhouses. Therefore, the microbial quality of carcass surfaces should be correlated with the standard operating protocols and hygiene practices of abattoirs (Wheatley et al., 2010).

Enteric bacteria (e.g. Gram-negative, particularly *Escherichia coli*) from beef cattle comprise the primary microbial contaminants of carcass surfaces (Ali et al., 2010). These bacteria can be pathogenic or non-pathogenic and can harbor antibiotic resistance traits that could be spread to the microbiota of people and other animals (Nys et al., 2004). *E. coli* is typically part of the fecal bacterial community in cattle and can acquire antibiotic-resistance traits quickly through horizontal gene transfer (Silva et al., 2014). It is also relatively simple to identify by using selective media. Thus, *E. coli* is often selected as a representative organism to evaluate microbial contamination because its susceptibility patterns reflect the diversity of resistance in a bacterial population (Medeiros et al., 2011).

From a broader perspective, antibiotic resistance is considered the 3<sup>rd</sup> major public health challenge of the 21<sup>st</sup> century (Spellberg et al., 2008). Food, including beef, can play an important role as a fomite for transmission of antibiotic resistant bacteria to people (Christopher et al., 2013). Studies in Tanzania have shown that among the 9% of children's death caused annually by pathogenic *E. coli*, at least 1/3<sup>rd</sup> of the pathogens were resistant to antibiotics (Huynh et al., 2015). Despite disease outbreaks caused by multidrug resistant (MDR) *E. coli* in children and surgical patients (Manyahi et al., 2014), little is known about the prevalence of antibiotic resistant enteric bacteria from food animal sources in Tanzania.

Therefore, we evaluated the microbial quality and prevalence of antibiotic resistant *E. coli* on beef carcass' surfaces. We also studied the impact of the size of the slaughter facilities and the carcass handling protocol in the prevalence of bacteria and antibiotic resistance on

the surface of beef carcass in the Arusha region of Tanzania.

## MATERIALS AND METHODS

MacConkey agar, Hi-Chrome agar and Luria-Bertani (LB) broth (Becton, Dickson and Company, Sparks, MD, USA) were used for bacterial enumeration, isolation and culture. Unless otherwise specified, incubation was overnight (16 - 18 h) at  $37^{\circ}$ C for all procedures. Antibiotic susceptibility patterns of all *E. coli* isolates were determined by agar dilution method with the minimum inhibitory concentration (MIC) breakpoints informed by Clinical and Laboratory Standards Institute guidelines (CLSI, 2014). The stock solutions of antimicrobials were prepared by mixing antibiotic powder with water or with dimethyl sulfoxide (DMSO, Becton, Dickson and Company, Sparks, MD, USA) and stored at 20°C.

## Sampling strategy

association between To examine the microbial surface contamination and sanitation procedures in abattoirs, the presence/absence of basic slaughter house facilities including slaughtering and carcass cleaning protocols were noted as recommended by Small Slaughterhouses and Meat Hygiene for Developing Countries (WHO publication VPH 83.56). The carcass surface swab samples were collected to determine the load of cultivatable Gram-negative bacteria including E. coli. Observations and samples were collected from five cattle slaughter facilities involving five sampling events between May and July, 2015 in Arusha region, Tanzania. The number of animals slaughtered at these facilities varied between 2 and >100 per day. Based on the number of animals slaughtered per day, these facilities were considered small- (2-10, sites A and B), medium- (18-25, sites C and D) and large- (>100, site E) scale facilities (Table 2). All facilities slaughtered animals between 3 am and 6 am. Carcasses were selected for sampling based on their availability. For this project, it is surmised that large and modernized slaughter facilities would maintain preferable slaughter and sanitary procedures resulting is less surface contamination to the meat.

#### Sample collection

Sterile, pre-moistened (with sterile water) 35 cm<sup>2</sup> gauze pads were used to swab approximately 100 cm<sup>2</sup> of surface area on two sides of each carcass immediately after slaughtering (small and medium scale) and in large-scale facilities immediately before carcasses were moved to the chilling facility (all other slaughter procedures were complete at this time). The moistened pads were prepared in a sterile 50 ml conical tube containing 15 ml of sterile water for Gram-negative bacteria (*E. coli*) as transport medium. After rubbing, the gauze pads were placed into the same sterile tube and transported (within 2 h of collection) to the laboratory for further processing in ice-cold boxes.

•• •• •		Positive samples (%)		Microbial load (Log <sub>10</sub> CFU/cm <sup>2</sup> ) Average (%) ±SE (95% confidence interval)		
Abattoir	Ν	Gram-negative bacteria <sup>1</sup>	E. coli	Gram-negative bacteria <sup>1</sup>	E. coli	
Small (2-10) <sup>#</sup>						
A	20	100	90	1.42 ± 0.26 (1.16-1.67)	1.05 ±0.32(0.73-1.36)	
В	20	100	85	1.36 ± 0.21 (1.15 -1.57)	0.94 ± 0.25(0.69-1.19)	
Average		100	87.5	1.39	0.99	
Medium (18-25) <sup>#</sup>						
С	25	100	76	1.53 ± 0.16 (1.37 - 1.69)	0.70 ± 0.21(0.49-0.91)	
D	20	95	70	1.02 ± 0.26 (0.76-1.28)	0.62 ± 0.26(0.37-0.88)	
Average		97.5	73	1.29	0.65	
P-value		0.5	0.069	0.66	0.0082	
Large (30 – 100) <sup>#</sup> E	40	100	95	1.77 ± 0.16 (1.61 - 1.92)	1.42 ± 0.16(1.25-1.58)	

**Table 2.** Distribution of Gram-negative and *E. coli* positive meat samples and the Log<sub>10</sub> colony forming units of total Gram-negative count and *E. coli* of the beef carcass' samples collected from five slaughter houses located in the vicinity of Arusha, Tanzania.

<sup>#</sup>Number of animals slaughtered per day. <sup>1</sup>Counts of all the bacteria grew on Mac Conkeyagar plates. SE– Standard error; CFU – colony forming units. N- number of swabs.

#### Enumeration and isolation of Escherichia coli

Swabs were mixed well in the transport media (sterile water) and approximately 300 µl of suspension was plated onto MacConkey agar plates with the help of sterile glass beads. The plates were airdried briefly and incubated overnight. After incubation, plates were examined for the presence of bacterial colonies that were then enumerated as the total number of Gram-negative bacteria. Colonies with morphology similar to E. coli (pink to dark rose lactose fermenting colonies) were enumerated separately and up to 12 isolates were picked using sterile tooth-picks and inoculated into 150 µl of Luria-Bertani broth within individual wells of 96-well assay plates. These presumptive E. coli isolates were confirmed for their identity by using Hi-Chrome agar. Colonies that exhibited morphology similar to E. coli on Hi-chrome agar were added with glycerol (15% final concentration vol/vol) and stored at -80°C for further characterization. Colonies that did not meet these selection criteria were not included in the analysis of E. coli isolates (Mwanyika et al. In press). E. coli counts were reported as colony forming units per cm<sup>2</sup> (cfu/cm<sup>2</sup>) of carcass surface (100 cm<sup>2</sup> equal to 15 mL of swab wash).

#### Determining antibiotic susceptibility

To determine the prevalence of antibiotic resistance up to 12 *E. coli* isolates from each sample were used with a breakpoint assay (Galland et al. 2001). Briefly, each isolate was tested against 11 antibiotics with their corresponding break point concentration listed by Clinical Laboratory Standards Institute (Tadesse et al., 2012). These were: Ampicillin (Amp, 32 µg/ml, VWR International LLC, Sanborn, NY, USA), amoxicillin (Amx, 32 µg/ml, MP BiomedicalsLLC, Solon, OH), ceftazdime (Ceft, 8 µg/ml, SIGMA-ALDRICH Co., St.Louis , MO), cefotaxime (Ctx, 8 µg/ml, Chem-Impex International INC, Wood Dale, IL), ciprofloxacin (Cip, 4 µg/ml, Enzo Life Sciences Inc, Farmingdale, NY), chloramphenicol (Chlo, 32 µg/ml, Mediatech Inc.), streptomycin (Str, 16 µg/ml, MP Biomedicals), tetracycline (Tet, 16 µg/ml, MP Biomedicals) and

trimethoprim (Tri, 8 µg/ml, MP Biomedicals).

MacConkey agar plates (150 mm diameter) were prepared with each antibiotic using the final concentrations described above. *E. coli* isolates from the 96-well plates were transferred onto the agar plates containing antibiotics by using a sterile 96-pin replicator. One susceptible (*E. coli* K-12) and two antibiotic resistant (*E. coli* NM-1 and NM-2, water isolates collected from Tanzania) strains were used as negative and positive controls, respectively for antibiotic susceptibility profiling of *E. coli* isolates. For this study, isolates resistant to  $\geq$  2 antibiotics were considered MDR.

#### Statistical analysis

Bacterial counts per sample and per slaughterhouse were reported as the mean  $\log_{10}$  cfu/cm<sup>2</sup> with 95% confidence intervals (CI). Binary coding was used to record the susceptible (0) or resistant (1) phenotypes for each isolate X antibiotic test. Data was processed by using MS Excel 2000 (Microsoft Corporation, Redmond, WA) and descriptive metrics were computed by using MS Access. Data was subsequently analyzed using R-statistical package (version 3.2.1) to determine (1) the differences in bacterial counts between abattoirs and (2) the differences in the prevalence of resistance between abattoirs and antimicrobials. One-way analysis of variance (ANOVA), Turkey HSD post-hoc multiple comparison test ( $\alpha = 0.05$ ) and Student t-tests were used to compare the differences between small and medium scale facilities. Due to the lack of replicates, the large-scale abattoir was not compared with other abattoir types.

# RESULTS

The largest abattoir ("E") was well equipped including hand-wash stations with hot running water and stations to sanitize knives. Animals were delivered from auctions by truck and were typically held in pens for 24 h before slaughter. The slaughter process at this facility was unidirectional, which has the advantage of limiting contact

Abattoir	Number of <i>E. coli</i> <sup>a</sup> collected	Average(%) ±SE;(95%CI) of ABR
Small (2- 10) <sup>#</sup>	432	
A	216	21 ± 0.09 (0.11 - 0.30)
В	216	19 ± 0.07 (0.13 - 0.27)
Average		21
Medium (18-25) <sup>#</sup>	396	
С	228	32 ± 0.12 (0.20 - 0.43)
D	168	28 ± 0.12 (0.16 - 0.40)
Average		31
P value		0.07
Large (30 – 100) <sup>#</sup> E	444	27 ± 0.06 (0.21 - 0.33)

**Table 3.** Prevalence of antibiotic resistant (ABR) *E. coli* isolated from the surface of beef carcasses in five cattle abattoirs located in Arusha district, Tanzania.

<sup>a</sup>Up to 12 isolates from each sample. *P*-value for the difference in average ABR between medium and small-scale abattoirs.

between initial slaughter waste and the finished products. Prior to storage in a cold room, carcasses were washed with hot water (no additives) and obvious contaminated areas were trimmed off. Routine inspections were conducted before carcasses were sent to a cold room. The inspection protocol was based on physical inspection for lesions typical of foot and mouth disease, bovine tuberculosis and cysticercosis with attention to the liver, heart, kidneys and lymph nodes.

Abattoirs A. B. C and D (small and medium) were located in areas with lower human population density. Most of the animals were from farmers in the immediate area and occasionally from auctions. Qualitatively, these facilities appeared to employ less stringent hygiene practices and lacked separate working areas, potable running water, and drainage systems; cold rooms were not available for storage. Two facilities (A and B) used small rivers as a source of water and workers cleaned themselves at the river after slaughtering animals. All slaughter processes at these facilities were performed on the floor in the same area where the finished product and initial slaughter wastes were in close physical proximity. Importantly, when the number of animals to be slaughtered exceeded the abattoir's handling capacity, especially on market days, cattle were slaughtered outside in open areas near the slaughterhouses. A sponge dipped in cold water was used to clean carcasses of visible contaminants before the products were removed from the abattoir. Only abattoir C in this category had meat inspectors to examine the carcasses on a regular basis.

# Prevalence and load of bacteria

A total of 125 surface swab samples collected from five different slaughter houses (40 each from small and large and 45 from medium; Table 2) were processed to estimate the prevalence and load of cultivable Gram-

negative bacteria including E. coli. From 125 samples, 1272 E. coli isolates were recovered (Table 3). Almost all (99.2%) of the carcass samples were positive for Gramnegative bacteria and 84.8% of the samples were positive for E. coli (Table 2). Detection of total Gramnegative bacteria and E. coli positive samples did not differ significantly between small- and medium-scale slaughterhouses (Table 2). Nevertheless, beef carcasses from both small- and medium-scale slaughter facilities harbored bacterial counts that were lower than the maximum recommended by FAO (Table 1) for total bacteria (<1.5 log cfu/cm<sup>2</sup>) and *E. coli* (<0.8 log cfu/cm<sup>2</sup>) (Table 2). Beef carcasses from the large slaughterhouse harbored more total bacteria (1.79 log cfu) and E. coli (1.44 log cfu) on their surfaces (Tables 1 and 2). With respect to FAO standards (Table 1), the E. coli load was satisfactory for 35.2% of individual carcasses while the remaining were considered excessively contaminated. If we assume that total bacterial counts from MacConkey agar (total Gram-negative bacteria) are the equivalent to the FAO standard for total bacteria (Table 1), then 47.2% carcasses were considered safe for human of consumption.

# Prevalence of antibiotic resistant *E. coli*

On average, antibiotic-resistant isolates of *E. coli* were more prevalent in medium  $(31\% \pm 0.08, \text{mean} \pm 95\% \text{ CI})$ as compared to small facilities  $(21\% \pm 0.06)$  (*P* = 0.07; Table 3). The large slaughterhouse also harbored an equal or higher percentage of resistant isolates as compared to medium facilities (Table 3). Among the *E. coli* isolates, Tet resistance was predominant in small (31%) and large (27%) houses followed by Amp resistance (42% and 11%; Table 4); in medium-scale facilities Amp resistance was predominant (25%) followed by Tet (11%) resistance. Resistance to Amx, Cip, Str, Sul

Slaughter size	Amp	Amx	Chl	Cip	Ctx	Gen	Str	Sul	Tet	Tri
Small	42 ± 6.5	0	0	0	6.5 ± 1.4	0	0	0	31 ± 1.3	0
А	13	0	0	0	0.4	0	0	0	37	0
В	71	0	0	0	12.5	0	0	0	25	0
Medium	25± 4	0	0	0	2 ± 0.4	1±0.2	0	0	11 ± 0.9	0
С	41	0	0	0	4	2	0	0	15	0
D	8.3	0	0	0	0	0	0	0	7	0
Large E	11	1	2.7	3	8	0	1	4	27	1

**Table 4.** Average prevalence (%) of antibiotic resistant *E. coli* collected from the surface of beef carcasses obtained from five different abattoirs in Arusha district, Tanzania. The mean ± standard error was listed for small and medium slaughterhouses.

and Tri was not found in *E. coli* isolated from small and medium facilities and resistance to ChI, Ctx and Gen was limited or nonexistent in those facilities. Except ChI and Gen resistance, *E. coli* isolated from the large facility harbored bacteria that were positive for all other tested resistance phenotypes (Table 4). *E. coli* isolates resistant for Cfz were not found in any of the samples tested. All of the multidrug resistant *E. coli* isolated in this study were resistant to 2-3 antibiotics. Among the 19 unique combinations of resistance traits, isolates from small houses harbored four (AmpTet) and medium-scale isolates harbored five phenotypes while the large slaughterhouse harbored 15 phenotypes (AmpTet-2% followed by ChISul-1.3% and others) (Table 5).

# DISCUSSION

For many countries Hazard Analysis Critical Control Point (HACCP) systems have become mandatory as a hygiene control strategy in meat processing plants (Çalicioğlu et al., 2010). An example of the benefit for adopting HACCP comes from South Africa, which has documented a significant increase in microbiological quality of beef carcasses following HACCP implementation with a commensurate economic benefit through expansion of meat markets (Govender et al., 2013). In Tanzania, food inspection still relies on a "see, smell and touch" protocol that does not detect microbiological and chemical contaminants (Ruteri, 2009). For this reason Tanzanian meat is regarded as unsafe despite having the third largest livestock production system in Africa (Ologhobo et al., 2010). Recently, the Tanzanian Food and Drug Authority shutdown several abattoirs in Arusha region after failed inspections. Presumably, the level of production is stretching the capacity of local abattoirs and increasing the likelihood that non-compliant practices are being used.

If we assume that total Gram-negative bacterial counts are equivalent to FAO standards for total bacteria on beef carcasses, then more than 50% of beef carcasses from the current study could be accepted for human consumption. The cool weather in Arusha might have

affected bacterial count because abattoirs used generally poor manufacturing practices. The remaining >45% of beef carcasses were excessively contaminated. While we cannot statistically evaluate the correlation between facility size and microbiological quality of meat, it is notable that the one large facility included in the current study had higher loads of bacteria and a greater diversity of antibiotic resistant E. coli. This was the case despite what appeared to be application of more effective sanitation control procedures. Consequently, the total number of animals being handled, or the pace by which they are processed, may be a more important risk factor for carcass contamination. Consistent with this hypothesis, a study from Alberta, Canada, found that high volume abattoirs had significantly higher (P<0.01) mean counts for coliforms and E. coli than low volume abattoirs (Bohaychuk et al., 2009).

Transportation and lairage effects could be another factor. Transportation can stress animals and increase defecation rates with correspondingly greater possibilities of hide contamination. Also cattle in the large abattoir were mixed with small ruminants in the holding pen and this could facilitate transmission of bacteria between animals. For example, one study found that detection of *Salmonella* increased from 18.5 to 47.7% with a 66 h increase in time that stock spent in lairage (Morgan et al., 1987). Others have reported that there is an increase in carcass bacterial counts associated with transportation (Engineer et al., 2008).

The higher prevalence of antibiotic resistant bacteria at the large facility could also be affected if their suppliers use more antibiotics (Nyenje and Ndip, 2013). For example, because their stock comes from larger suppliers, high-density husbandry increases the chances of disease transmission and presumably increases the demand or need for antimicrobials to sustain their herd health.

The medium volume facilities examined in this study routinely hung carcasses soon after slaughtering, which could explain a reduced bacterial load. Carcass hanging was not observed at the two small-scale abattoirs. In Nigeria, meat from small abattoirs had mean *E. coli* counts of 4.2 log cfu/g (Iroha et al., 2011). In Brazil,

AMR phenotypes <sup>a</sup>	Small	Medium	Large	
None	65.17 ± 11.5	36.25 ± 15.4	54.7	
Amp	26.25 ± 15.4	20.46 ± 14.2	8.33	
Amx	0	0	0.23	
Cfd	0	0.21 ± 0.21	0	
Chm	0	0	0.43	
Сір	0.21 ± 0.21	0.83 ± 0.83	0	
Ctx	4.38 ± 3.96	$0.5 \pm 0.5$	6.62	
Gen	0	$0.7 \pm 0.5$	0	
Sul	0	0	0.64	
Tet	17.29 ± 17.29	8.29 ± 4.62	22.22	
AmpCtx	$2.08 \pm 2.08$	1.33 ± 1.33	0	
AmpGen	0	0.17 ± 0.17	0	
AmpTet	2.17 ± 0.5	11.46 ± 9.38	1.92	
AmxChm	0	0	1.92	
AmxTet	0	0	1.92	
ChmSul	0	0	1.28	
CtxTet	0	0	0.85	
SulTet	0	0	0.43	
AmpGenTet	0	0.21 ± 0.21	0	
AmxChmTet	0	0	0.21	
ChmSulTet	0	0	0.21	
SulTetTri	0	0	0.21	
AmpAmxStrSul	0	0	0.21	
AmpChmStrTri	0	0	0.21	
AmpChmSulTet	0	0	0.21	
AmpStrSulTri	0	0	0.21	
AmpSulTetTri	0	0	0.21	
AmpStrSulTetTri	0	0	0.21	

**Table 5**. Average prevalence (%) of antibiotic resistant phenotypes among the *E. coli* isolates obtained from small, medium and large-sized slaughterhouses in the Arusha region.

<sup>a</sup>Amp = ampicillin; Amx = amoxicillin; Cfd = ceftazidime; Chm = chloramphenicol; Cip = ciprofloxacin; Ctx = cefotaxime; Gen = gentamycin; Str = streptomycin; Sul = sulfamethoxazole; Tet = tetracycline; Tri = trimethoprim.

Brazil, carcass surfaces in a small abattoir had a total viable bacterial count ranging between 1.78 and 2.78  $\log_{10}$  cfu/cm<sup>2</sup> (Caselani et al., 2013). Importantly, abattoir layout can have a significant impact via the distribution of air-borne contaminants (Prendergast et al., 2004).

Resistance to ampicillin and tetracycline were the most common phenotypes found in our study. Another study reported that tetracycline resistance was frequently detected among *E. coli* in South African cattle, swine and people (Ateba and Bezuidenhout, 2008). Tetracycline resistance was evident for *Salmonella, E. coli and Campylobacter* found in meat from Canada (Cook et al., 2009). The wide distribution of these resistance traits is favored by the location of genes on mobile genetic elements such as plasmids and transposons (Schnabel and Jones, 1999). Use of tetracycline antibiotics in livestock farming is relatively common as compared to other classes of antibiotics and a recent study in Tanzania found that 70% of meat was positive for tetracycline residues (Darwish et al., 2013). Consequently, use of antibiotics in livestock may drive the higher prevalence of these resistance traits in enteric bacteria.

Importantly, very limited resistance to third-generation cephalosporines (Ctx, Cfz), was detected considered drugs of last resort for the treatment of food-borne pathogens like *Salmonella* and *Shigella* (Xia et al., 2011). Resistance to chloramphenicol was also very limited (1%). We conducted an informal survey of veterinary drug vendors in the Arusha area and mostly found oxytetracycline, penicillin-streptomycin and tylosin products (data not shown). There was no evidence that vendors carried either cephalosporins or chloramphenicol products for use in food animals. Low levels of resistance to chloramphenicol have also been reported in other studies (Tadesse et al., 2012; Kibret and Tadesse, 2013). Importantly, several countries have banned the use of chloramphenicol in food animal production, these include the USA, Canada and South Africa (Berendsen et al., 2010; Ting et al., 2001).

In Ifakara Tanzania (Vila et al., 1999), multidrug resistant *E. coli* was associated with 38% of diarrhea in children from a rural community. Another study showed that the rate of bacteremia in children was 13.9% and one third of these children presumably died due to failure of treatment associated with multidrug resistant bacteria of which Gram-negative bacteria were the leading cause (43.5%) (Blomberg et al., 2007). Interestingly, beef may not be a major source of these isolates given that for the current study only 4.6% of *E. coli* were resistant to  $\geq 2$  antibiotics. In contrast, a high prevalence of resistant bacteria has been reported from raw milk from cattle and from other food animals including broiler chickens (Nonga et al., 2010; Lubote et al., 2014).

It is important to note that beef demand in Tanzania is increasing with the increase in human population, but it is questionable if the production system is equipped to deal with a rapid pace of change. Diseases like "Ndigana and Kizunguzungu" (anaplasmosis and ehrlichiosis, respecttively) and East Coast Fever are significant challenge to farmers in northern Tanzania (Halliday et al., 2015). Their prevention and treatment promote the reliance on antimicrobials (including oxytetracycline) and this may contribute to selection for antibiotic resistant organisms.

Clearly, less microbial surface contamination is possible and should be the goal for the industry in Tanzania. For example, whereas the vast majority of carcasses in the current study were contaminated with *E. coli*, this was only true for 7.6% beef carcasses in Irish abattoirs (McEvoy et al., 2003) while only 3% of retail meat samples were positive for *Salmonella* in Washington, D.C (White et al., 2001). Beef carcasses from the Arusha area have limited contamination from multidrug-resistant *E. coli*. This study provides a baseline to consider how this prevalence may change with population growth in this region.

# **Conflict of Interests**

The authors have not declared any conflict of interests.

# Abbreviations

**ABR**, Antibiotic resistance; **FAO**, Food and Agriculture Organization; **HACCP**, Hazard Analysis Critical Control Point; **MDR**, multi-drug resistance; **WHO**, World Health Organization.

## REFERENCES

Ali NH, Farooqui A, Khan A, Khan AY, Kazmi SU (2010). Microbial contamination of raw meat and its environment in retail shops in Karachi. Pak. J. Infect. Dev. Ctries. 4(6):382-388.

- Ateba CN, Bezuidenhout CC (2008). Characterisation of *Escherichia coli* O157 strains from humans, cattle and pigs in the North-West Province, South Africa. Int. J. Food. Microbiol. 128(2):181-188.
- Berendsen B, Stolker L, Jong J, Nielen M, Tserendorj E, Sodnomdarjaa R, Cannavan A, Elliott C (2010). Evidence of natural occurrence of the banned antibiotic chloramphenicol in herbs and grass. Anal. Bioanal. Chem. 397(2):1955-1963.
- Blomberg B, Manji KP, Urassa WK, Tamim BS, Mwakagile DM, Jureen R, Msangi V, Tellevik MG, Holberg-Petersen M, Harthug S (2007). Antimicrobial resistance predicts death in Tanzanian children with bloodstream infections. BMC Infect. Dis. 7(1):1-14.
- Bohaychuk VM, Gensler GE, Barrios PR (2009). Microbiological baseline study of beef and pork carcasses from provincially inspected abattoirs in Alberta, Canada. Can. Vet. J. 52(10):1095-1100.
- Çalicioğlu M, Buege DR, Luchansky JB (2010). Effect of preevisceration, skin-on carcass decontamination sanitation strategies for reducing bacterial contamination of cattle during skinning. Turk. J. Vet. Anim. Sci. 34(3):261-266.
- Caselani K, Prata LF, Prata CB, Bizari PA, Pereira GT (2013). Relationship between HACCP controls, pathogen reduction program and meat quality during cattle slaughter process. Rev. Port. Cienc. Vet. 108(585):29-38.
- Christopher A, Mshana SE, Kidenya BR, Hokororo A, Morona D (2013). Bacteremia and resistant gram-negative pathogens among underfives in Tanzania. Ital. J. Pediatr. 39(2):27-34
- CLSI (Clinical and Laboratory Standards Institute) (2014). Performance standards for antimicrobial susceptibility testing; twenty-forth informational supplement. CLSI document M100-S24 Clinical and laboratory standards institute, Wayne, Pennsylvania, USA. 34:1.
- Cook C, Reid-Smith R, Irwin R, McEwen SA, Valdivieso-Garcia A, Ribble C (2009). Antimicrobial resistance in Campylobacter, Salmonella, and Escherichia coli isolated from retail turkey meat from southern Ontario, Canada. J. Food. Prot. 72(3):473-481.
- Darwish WS, Eldaly EA, El-Abbasy MT, Ikenaka Y, Nakayama S, Ishizuka M (2013). Antibiotic residues in food: the African scenario. Jpn. J. Vet. Res. 61(6):13-22.
- De Filippis F, La Storia A, Villani F, Ercolini D (2013). Exploring the sources of bacterial spoilers in beef steaks by culture-independent high-throughput sequencing. PLoS One 8:e70222.
- Engineer A, Berry ED, Wells JE, Arthur TM (2008). The impact of stress level on fecal bacteria and pathogen shedding in feedlot cattle. Food. Microbiol. 3(8):34-40.
- Food and Agriculture Organization (FAO) (2013). Microbiological Guidelines for Food; Meat processing technology for small and medium scale producers. FAO document M25 Food and Agriculture Organization, Rome, Italy. pp. 344-348.
- Galland JC, Hyatt DR, Crupper SS, Acheson DW (2001). Prevalence, Antibiotic Susceptibility, and Diversity of Escherichia coli O157: H7 Isolates from a Longitudinal Study of Beef Cattle Feedlots Prevalence. Appl. Environ. Microbiol. 67(4):1619-1627.
- Govender R, Naidoo D, Buys EM (2013). Managing Meat Safety at South African Abattoirs. Proceedings of World Academy of Science, Engineering and Technology 76:747-752.
- Halliday JB, Allan KJ, Ekwem D, Cleaveland S, Kazwala RR, Crump JA (2015). Endemic zoonoses in the tropics: a public health problem hiding in plain sight. Vet. Rec. 176(9):220-225.
- Heiman KE, Mody RK, Johnson SD, Griffin PM, Gould LH (2015). Escherichia coli O157 Outbreaks in the United States, 2003-2012. Emerg. Infect. Dis. 21(8):1293-1301.
- Huynh BT, Padget M, Garin B, Herindrainy P, Kermorvant E, Watier L, Guillemot D, Delarocque E (2015). Burden of bacterial resistance among neonatal infections in low income countries. BMC Infect. Dis. 15(1):1-13.
- Iroha IR, Ugbo EC, Ilang DC, Oji E, Ayogu TE (2011). Bacteria contamination of raw meat sold in Abakaliki, Ebonyi State Nigeria. J. Public Health Epidemiol. 3(1):49-53.
- Kibret M, Tadesse M (2013). The bacteriological safety and antimicrobial susceptibility of bacteria isolated from street-vended white lupin (Lupinus albus) in Bahir Dar, Ethiopia. Ethiop. J. Health Sci. 23(6):19-26.
- Lubote R, Shahada F, Matemu A (2014). Prevalence of Salmonella spp. and Escherichia coli in raw milk value chain. Am. J. Res. Commun.

2(9):1-13.

- Manyahi J, Matee MI, Majigo M, Moyo S, Mshana SE, Lyamuya EF (2014). Predominance of multi-drug resistant bacterial pathogens causing surgical site infections in Muhimbili national hospital, Tanzania. BMC Res. Notes 7(500):1-7.
- McEvoy JM, Doherty AM, Sheridan JJ, Blair IS, McDowell DA (2003). The prevalence of Salmonella spp. in bovine faecal, rumen and carcass samples at a commercial abattoir. J. Appl. Microbiol. 94(4):693-700
- Medeiros MN, Oliveira DN, Rodrigues DP, Freitas DC (2011). Prevalence and antimicrobial resistance of Salmonella in chicken carcasses at retail in 15 Brazilian cities. Rev. Panam. Salud. Pública. 30(6):555-560.
- Morgan IR, Krautil FL, Craven JA (1987). Effect of time in lairage on caecal and carcass Salmonella contamination of slaughter pigs. Epidemiol. Infect. 98(4):323-330.
- Mwanyika G, Call DR, Rugumisa B, Luanda C, Mrutu R, Subbiah M, Buza J (2016). Load and prevelance of antimicrobial-resistant Escherichia coli from fresh goat meat in Arusha Tanzania. J. Food. Prot. In Press.
- Nonga HE, Simon C, Karimuribo ED, Mdegela RH (2010). Assessment of antimicrobial usage and residues in commercial chicken eggs from smallholder poultry keepers in Morogoro municipality, Tanzania. Zoonoses Public Health 57(5):339-344.
- Nyenje ME, Ndip RN (2013). The challenges of foodborne pathogens and antimicrobial chemotherapy : A global perspective. Afr. J. Micro Res. 7(14):1158-1172.
- Nys S, Okeke IN, Kariuki S, Dinant GJ, Driessen C, Stobberingh EE (2004). Antibiotic resistance of faecal Escherichia coli from healthy volunteers from eight developing countries. J. Antimicrob. Chemother. 54(5):952-960.
- Ologhobo AD, Omojola AB, OfongoST, Moiforay S, Jibir M (2010). Safety of street vended meat products-chicken and beef suya. Afr. J. Biotechnol. 9(7):4091-4095.
- Prendergast DM, Daly DJ, Sheridan JJ, McDowell DA, Blair IS (2004). The effect of abattoir design on aerial contamination levels and the relationship between aerial and carcass contamination levels in two Irish beef abattoirs. Food. Microbiol. 21(5):589-596.
- Ruteri JM (2009). Supply chain management and challenges facing the food industry sector in Tanzania. Int. J. Bus. Manage. 4(12):70-78.
- Xia S, Bianli X, Lili H, Jia-Yong Z, Lu R, Jin Z, Haomin C, Chaiwat P, Srirat P, Frank M, Aerestrup, Rene S (2011). Prevalence and characterization of human Shigella infections in Henan Province, China, in 2006. J. Clin. Microbiol. 49(1):232-242.

- Silva FP, Horvath MB, Silveira JG, Pieta L, Tondo EC (2014). Occurrence of Salmonella spp. and generic Escherichia coli on beef carcasses sampled at a brazilian slaughterhouse. Braz. J. Microbiol. 45(1):17-24.
- Sousa CP (2008). The impact of food manufacturing practices on food borne diseases. Braz. Arch. Biol. Technol. 51(4):615-623.
- Spellberg B, Guidos R, Gilbert D, Bradley J, Boucher HW, Scheld WM, Bartlett JG, Edwards J (2008). The epidemic of antibiotic-resistant infections: a call to action for the medical community from the Infectious Diseases Society of America. Clin. Infect. Dis. 46(1537):155-164.
- Tadesse DA, Zhao S, Tong E, Ayers S, Singh A, Bartholomew MJ, McDermott PF (2012). Antimicrobial drug resistance in Escherichia coli from humans and food animals, United States, 1950–2002. Emerg. Infect. Dis. 18(5):741-749.
- Ting L, Peter W, KefeiW (2001). Simple and Rapid Analysis of Chloramphenicol in Milk by LC-MS / MS. Thermo. Fish. Sci. 4(407):5-8.
- Vila J, Vargas M, Casals C, Urassa H, Mshinda H, Schellemberg D, Gascon J(1999). Antimicrobial Resistance of Diarrheagenic Escherichia coli Isolated from Children under the Age of 5 Years from Ifakara, Tanzania. Antimicrob. Agents Chemother. 43(12):3022-3024.
- Wheatley P, Giotis ES, McKevitt AI (2010). Effects of slaughtering operations on carcass contamination in an Irish pork production plant. Ir. Vet. J. 4(6):382-388.
- White DG, Zhao S, Sudler R, Ayers S, Friedman S, Chen S, McDermott PF, McDermott S, Wagner DD, Meng J(2001). The isolation of antibiotic-resistant Salmonella from retail ground meats. N. Engl. J. Med. 345(16):1147-1154.