Prevalence of antibiotic resistant *Salmonella* isolated from beef in Arusha region, Northern Tanzania

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Abstract

Antibiotic-resistant *Salmonella* are frequently isolated from people in Tanzania and this highlights the potential contribution that food-animal agriculture could play in the emergence and dissemination of resistant *Salmonella.* Consequently, we investigated the prevalence of *Salmonella* and their antibiotic resistance phenotypes from beef in the Arusha region. Samples were then enriched in Tryptic soy broth (TSB; 35°C for 24 h). A total of 351 *Salmonella* were isolated from these enrichments by plating onto Xylose lysine deoxycholate (XLD) agar. An agar dilution assay was used to screen for resistance to 11 antibiotics. *Salmonella* was recovered from most carcasses (94%) and the prevalence of antibiotic resistant isolates did not differ between medium (n=2) and small (n=2) slaughterhouses, but the majority of isolates 189 (53.9%) were resistant to at least one antibiotic agent. Only 13.5% were resistant to two or more antibiotics (mostly ampicillin and trimethoprim resistance). For individual antibiotics, resistance was most prevalent to trimethoprim (19.4%), followed by ampicillin (6.3%) and amoxicillin (5.1%). All *Salmonella* isolates were sensitive to gentamycin (100%) and most isolates (99.7%) were sensitive to chloramphenicol. The high prevalence of *Salmonella* from our samples indicates that beef from the Arusha region is a potentially important source of transmission to people. Relative to other localities and food sources, relatively few *Salmonella* isolates were antibiotic resistant.

Efforts should be directed to improve abattoir sanitation and to educate the public about potential hazards from handling and consuming uncooked beef.

Keywords: *Salmonella*, antibiotic resistance, slaughterhouse hygiene, meat contamination, public health.

{**Citation:** Rehema Murutu. Prevalence of antibiotic resistant *Salmonella* isolated from beef in Arusha region, Northern Tanzania. American Journal of Research Communication, 2016, 4(7): 1-16} www.usa-journals.com, ISSN: 2325-4076.

Introduction

Salmonellosis is a major public health challenge in developing countries (*1*). Contaminated animal foods are considered an important route of *Salmonella* transmission to people *(2,3)*. For example, in 2009, 59/156 (38%) clinical cases of childhood Salmonellosis were confirmed by rural Tanzania hospitals *(4)* (far more were probably undetected). In 2012, 39% of 346 febrile adult patients were infected with *Salmonella* in northwestern Tanzania *(5)*. Furthermore, there has been a recent "spike" in the number of patients with gastrointestinal illness in Northern Tanzania *(6)*. Because most bugs causing diarrhea are food borne *(7)*, there is a need to understand the microbial quality of meat especially with pathogenic bacteria like *Salmonella* given the increasing rate of meat consumption in the region. Such estimates could enable us to appreciate the level of contamination and the comparative importance of beef as potential sources of *Salmonella* infections *(8)*.

Cattle in northern Tanzania routinely receive oxytetracycline injections to prevent and treat diseases including mastitis and Contagious Bovine Pleuropneumonia (CBPP), both of

which are endemic to the region *(9,10)*. This practice is thought to promote antibiotic resistance in intestinal microflora including *Salmonella*. Contaminated meat can act as a fomite for transfer of *Salmonella* to people where the problem is exacerbated by poor maintenance of hygiene-related infrastructure and problems in implementation of control measures, especially in slaughter houses *(11).*

Abattoirs have been associated with meat contamination and outbreaks of meat-borne pathogens *(12)*. In Arusha, Tanzania, there is a large number of small abattoirs that have grown as a cottage industry to accommodate the growing demand for meat. In most scenarios, these premises are not equipped to minimize microbial contamination. Even a single large abattoir in the study does not employ Hazard Analysis Critical Control Point (HACCP) practices and the facility is highly congested, casting serious doubt on the hygienic quality of the products. Elsewhere in the world, poor slaughter methods are known to expose people to infections by *Salmonella* and other pathogens *(13)*.

To date there has been no systematic assessment of the occurrence of *Salmonella* on beef carcasses in northern Tanzania. Here we qualitatively evaluate the slaughter and cleaning programs at five slaughterhouses and compare them with the occurrence of *Salmonella* and distribution of antibiotic resistant (ABR) and multi-drug resistant (MDR) *Salmonella*.

Materials and Methods

Primary reagents: Tryptic soy broth (TSB), Xylose lysine deoxycholate (XLD), triple-sugar-iron (TSI), Luria-Bertani (LB) and MacConkey agar (Becton, Dickson and Company, Sparks, MD, USA) were used for bacterial enrichment, isolation and sensitivity test.

Sample collection: Over a period of four weeks between July and August, 2015, five slaughterhouses, located in three different districts of Arusha City, were visited once a week. At each visit processing practices were observed and beef carcasses were swabbed by use of sterile gauze pad *(14)*, pre-wetted with 20 ml of TSB. A total of 125 swab carcass samples were collected; high- (40 swabs), medium- (45 swabs) and low- (40 swabs) volume abattoirs. All samples were collected in the morning after completion of slaughter. Samples were maintained on ice, transported to the laboratory, and processed within 2 h of collection.

Prevalence and isolation of *Salmonella* on the surface of beef carcasses: A broth-enrichment technique was used to enhance detection sensitivity for *Salmonella***.** Each swab was immersed in 15 ml of TSB within a sterile 50 ml conical tube and incubated 24 h at 35°C. The following day the swab enrichments were mixed well and a loop-full of enriched culture was spread on XLD agar and incubated at 37°C for 24 h. The colonies with transparent red halo and black center were considered putative *Salmonella* isolates. These were transferred to TSI agar slant and incubated at 37°C for 24 h. Typical reactions for *Salmonella* to TSI were used to confirm the presence of *Salmonella (15)*. Up to three isolates were retained from each positive sample for a repository of 351 *Salmonella* isolates that were maintained in the 96-well assay plate containing LB broth with glycerol at -80°C (150 µl per well).

Antibiotic susceptibility determination: Antibiotic susceptibility of all *Salmonella* isolates was determined by agar dilution method with minimum inhibitory concentration (MIC) breakpoints informed by Clinical and Laboratory Standards Institute guidelines *(16)*. Each *Salmonella* isolate was tested for resistance by using a 96-pin replicator to transfer isolates from archive plates onto

MacConkey agar plates (150 mm diameter) supplemented with one of the following antibiotics: 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, Medaitech Inc., Manassas, VA), gentamycin (Gen, 32 µg/ml, Mediatech Inc.), streptomycin (Str, 16 µg/ml, AmrescoInc., Solon, OH), sulfamethoxazole (Sul, 512 µg/ml, MP Biomedicals), tetracycline (Tet, 16 µg/ml, MP Biomedicals), and trimethoprim (Tri, 8 µg/ml, MP Biomedicals). 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. 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. The NM-1 isolate was resistant to Amp, Amx, Cfz, Ctx, Cip, and Chl while NM-2 was resistant to Gen, Str, Sul, Tet and Tri. These strains were added manually to each agar plate. Plates were left open at room temperature for 20 min to dry and then incubated overnight at 37°C. After incubation the numbers of resistant isolates (those that grew) were enumerated and the average prevalence of resistant *Salmonella* for each antibiotic per samples was calculated. For this study, isolates resistant to ≥2 antibiotics were considered multidrug resistant (MDR).

Statistical analysis

The frequency of occurrence of *Salmonella* per sample and per slaughterhouse was reported as the average prevalence with 95% confidence intervals (CI). Antibiotic growth data was coded as

binary with susceptible (0) or resistant (1) phenotypes for each tested antibiotic. All the data were entered into MS Excel 2000 (Microsoft Corp., Redmond, WA) and were subsequently analyzed using R-statistical package (version 3.2.1) to determine (1) the differences in bacterial prevalence between abattoirs, and (2) the differences in the prevalence of resistance between abattoirs and antimicrobials. Descriptive and quantitative analyses were computed by using MS Access (Microsoft Corp.).

Results

Prevalence of *Salmonella.* A total of 351 *Salmonella* isolates were obtained including 120 from high-, 114 isolates from medium-, and 117 isolates from low-volume abattoirs (Table 1). *Salmonella* was detected for 95 to 100% of samples from individual slaughterhouses. We were more likely to detect *Salmonella*-positive samples from medium volume slaughterhouses compared with small volume facilities although the difference was not statistically significant (P=0.06, Student's t-test, df=1).

Prevalence of antibiotic resistant *Salmonella*: Of the 351 isolates from all abattoirs, 26.9% were resistant to ≥1 antibiotic and over half of these resistant isolates were obtained in medium-scale abattoirs with trimethoprim (58%) being the most prevalent (Table 2). A total of 189 (53.85%) isolates; 11.9% (Large), 24% (medium) and 18.2% (small) were resistant to only one antimicrobial (usually trimethoprim, ampicillin, sulfamethoxazole or ciprofloxacin).

Table 1. Distribution of *Salmonella*-positive meat samples collected from five slaughterhouses located in Arusha, Tanzania

Abattoir	N	% Occurrence of *Salmonella*
Small (2-10)[#]	40	
A	20	100
B	20	95
Average		97.5
Medium (18-25)[#]	45	
C	25	72
D	20	100
Average		86
***P*-value[a]**		**0.56**
Large (30 – 100)[#]E	40	100

[#] Number of animals slaughtered per day; N=number of swabs; [a]*P*-value calculated using Student's t-test.

Table 2. Average prevalence of antibiotic resistant (ABR) *Salmonella* isolated from the surface swab samples of beef carcasses slaughtered in five cattle abattoirs located in Arusha, Tanzania

Abattoir	N	Number of *Salmonella* isolated*	Average (%) ± SE of ABR
Small (2- 10)[#]	40	117	
A	20	60	47 ± 0.08 (0.41 - 0.52)
B	20	57	33 ± 0 (0.33 - 0.33)
Average			46
Medium (18-25)[#]	45	114	
C	25	54	50 ± 0.09 (0.43 - 0.57)
D	20	60	44 ± 0.07 (0.40 - 0.49)
Average			48
***P*-value[a]**			**0.64**
Large (30 – 100)[#]E	40	120	46± 0.05 (0.39 - 0.51)

N=number of swabs; ABR – Antibiotic resistance; SE – Standard error; [a]*P*-value calculated using Student's t-test.; *****Up to 3 isolates from each sample; [#] Number of animals slaughtered per day.

Salmonella isolated from medium-volume abattoirs harbored resistance to 10 of the 11 tested antibiotics, whereas isolates from low-volume abattoirs only harbored resistance to trimethoprim (10.3%) (Table 3). No isolates were resistant to gentamycin.

Table 3. Average prevalence (%) of antibiotic resistant *Salmonella* detected on the surface of beef carcasses obtained from different slaughter houses in Arusha district, Tanzania

Slaughterhouses		Amp	Amx	Ctx	Cfz	Cip	Chl	Str	Sul	Tet	Tri
Small		0	0	0	0	0	0	0	0	0	10 ± 3
	A	0	0	0	0	0	0	0	0	0	13
	B	0	0	0	0	0	0	0	0	0	7
Medium		7 ± 4	9 ± 2	9.5 ± 9.5	7 ± 4	10 ± 7	1 ± 1	4.5 ± 0.5	10 ± 5	7.5 ± 5.5	23 ± 8
	C	11	11	19	11	17	2	4	15	13	31
	D	3	7	0	3	3	0	5	5	2	15
Large	E	12	7	3	4	0	2	5	5	1	0

The mean ± standard errors were listed for low- and medium-volume facilities.

All MDR isolates (13.4%) were isolated from medium- and high-volume abattoirs. A total of 29 unique MDR *Salmonella* phenotypes were identified (19 in medium- and 12 in high-volume facilities) with resistance to ampicillin and trimethoprim being the most common for *Salmonella* isolated from both medium- (4.6%) and high- (5.8%) volume abattoirs (Table 4).

Table 4. Average (%) prevalence of different antibiotic resistance phenotypes among the *Salmonella* isolates obtained from different slaughterhouses in Arusha region

Antibiotic[a]	Small N=1[b]	Medium N=24	Large N=18
None	52.5 ± 0.1	42 ± 2.46	78
Amp	0	10 ± 1.41	11.67
Amx	0	1.76 ± 0.02	0.83
Ctx	0	0.84 ± 0.18	0.83
Cfd	0	1.76 ± 0.02	0.83
Cip	0	0.93 ± 0.2	0
Chl	0	13.7 ± 2.19	1.67
Str	0	6.2 ± 0.25	5
Sul	0	9.9 ± 1.03	5
Tet	0	7.32 ± 1.19	0.83
Tri	10.35 ± 0.78	25 ± 2.13	25
AmpAmx	0	-	0.83
AmpTri	0	4.63 ± 1.04	5.83
AmxTri	0	1.85 ± 0.41	1.67
CfdCip	0	-	0.83
CfdCtx	0	1.85 ± 0.41	-
CfdTet	0	0.93 ± 0.21	-
CipTri	0	0.84 ± 0.19	-
CtxSul	0	0.93 ± 0.21	-
StrSul	0	0.84 ± 0.19	-
StrTri	0	-	0.83
TetTri	0	0.84 ± 0.19	-
AmpAmxCtx	0	0.84 ± 0.19	-
AmpAmxTri	0	-	1.67
AmxCfdTet	0	0.93 ± 0.21	-

AmxSulTri	0	2.69 ± 0.23	-
CfdCipTri	0	-	0.83
CipStrSul	0	-	0.83
CipSulTri	0	0.84 ± 0.19	-
StrSulTri	0	-	0.83
AmpAmxStrTri	0	-	0.83
AmpCfdCipTri	0	0.93 ± 0.21	-
CipCtxSulTet	0	0.93 ± 0.21	-
AmpAmxStrSulTri	0	-	0.83
CfdCipCtxSulTri	0	0.93 ± 0.21	-
CfdCipStrSulTet	0	-	0.83
CfdCtxSulTetTri	0	0.93 ± 0.21	-
CfdCipCtxStrTetTri	0	0.93 ± 0.21	-
CfdCipChmCtxSulTetTri	0	0.93 ± 0.21	-
AmxCfdCipCtxStrSulTetTri	0	0.93 ± 0.21	-

[a]Amp = ampicillin; Amx = amoxicillin; Cfd = ceftazdime; Chm = chloramphenicol; Cip = ciprofloxacin; Ctx = cefotaxime; Gen = gentamycin; Str = streptomycin; Sul = sulfamethoxazole; Tet = tetracycline; Tri = trimethoprim

[b]N = Number of phenotypes

Discussion

In this study a high prevalence of *Salmonella* was detected across all the abattoirs; medium (86%) < low (97.5%) < high (100%). Because *Salmonella* are considered to be pathogenic and have a very small infectious dose (15-20 cells; *(17)*), their presence in food is a significant potential risk to public health. Normally, cattle become contaminated with *Salmonella*

at the farm or during transportation *(18)*. Cross-contamination may continue during processing in the slaughterhouse, which is a key point in the production chain to limit the overall dissemination of *Salmonella (19)*. Cross contamination may result from intestinal carriage of *Salmonella* in the cattle, but also from contact with contaminated carcasses or surfaces in the abattoir *(20, 21)*.

Others have reported high recoveries of *Salmonella* but well below the prevalence found in the current study. For example, in Burkina Faso about 52% of beef carcasses were positive for *Salmonella (22)*, while in Dakar, Senegal, *Salmonella* was recovered from 43% of tested carcasses *(23)*. In this respect, the facilities examined for the current study are unique and the question remains if the high prevalence in Arusha can be correlated with hygiene and processing practices or due to other factors that are unique to northern Tanzania.

From our observations, medium and small slaughter facilities employed only very limited hygiene practices. Abattoir cleaning and disinfection procedures were not conducted because facilities were lacking. Cattle in the one large abattoir, however, were slaughtered using more rigorous hygienic procedures and carcasses were decontaminated using hot water (no additives). Despite the difference in slaughter procedure all the carcasses were contaminated with *Salmonella*. We speculate that the favorable climate of Arusha might have contributed to the high prevalence of *Salmonella* on meat carcasses *(24)*.

Resistance to trimethoprim (19.37%), ampicillin (6.27%), amoxicillin (5.13%) and sulfamethoxazole (4.84%) was evident from the current study. These antibiotics are widely used to treat bacterial infections in both people and animals. Amoxicillin and co-trimoxazole (sulfamethoxazole trimethoprim) are highly prescribed in Tanzanian hospitals to treat a variety of bacterial infections *(25)*. The presence of resistance to these antibiotics could be related to

local selection pressure (antibiotic use) or the emergence of resistant clonal strains that are successfully disseminated between and within populations *(26)*.

Notably, a relatively small proportion (13.5 %) of isolates were MDR as compared to other food animals in the literature *(27, 28)*, which also reports that MDR isolates tend to be associated with higher volume abattoirs. Our data together with those obtained by *(29)* demonstrate that resistance to ampicillin and trimethoprim or amoxicillin, sulfa and trimethoprim are the most common MDR phenotypes in this sampled area. These resistance combinations have been isolated in many African countries *(30, 31)*. A previous study from Tanzania showed that these antibiotics were frequently used in food-animal production with beta-lactams being used most frequently *(32)*. The same pattern has been reported for Kenya, Nigeria and South Africa *(33)*.

A number of isolates from high- and medium- volume slaughterhouses were resistant to the third-generation cephalosporins, cefotaxime and ceftazidime. This is an important public health concern because the development of cephalosporin-resistant *Salmonella*, particularly those of beef origin *(34)*, has been associated with outbreaks *(35)*. Several studies show that the prevalence of resistance to cefotaxime and ceftazidime and decreased susceptibility to other cephalosporins has increased among *Salmonella* isolated from food animals *(36)*. We detected little resistance to ciprofloxacin and gentamycin (99.7% and 100%, respectively). This finding contrasts with previous reports from Denmark and Senegal *(37, 38)*.

Acknowledgement

The authors acknowledge the Paul G. Allen School for Global Animal Health and the National Science Foundation (DEB1216040) for supporting part of this work.

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