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Vol. 10(11), pp. 348-356, 21 March, 2016
DOI: 10.5897/AJMR2015.7880
Article Number: BF81BA657723
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Surface waters in northern Tanzania harbor fecal coliform and antibiotic resistant *Salmonella* spp. capable of horizontal gene transfer

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Received 10 December, 2015; Accepted 18 February, 2016

Some *Salmonella* spp. are important pathogenic bacteria that can be transmitted to people via food and water and that can cause disease characterized by mild to severe enteric and systemic illness. In developing countries, infections caused by pathogenic antibiotic resistant *Salmonella* spp. are a major health challenge, particularly in children. Through the use of membrane filtration and Environmental Protection Agency (EPA) 1200 methods, the presence of fecal coliforms and antibiotic resistant *Salmonella* spp. in surface water sources was investigated, some of which had shared access for animals and people. Out of 100 water samples, 76 were positive for fecal coliforms and 63% of the positive samples contained >100 CFU / 100 ml of water. We observed a significant positive correlation between the number of fecal coliforms and the presence of *Salmonella* spp. (r=0.46, n=100, P=0.01). Importantly, >26% of the samples were positive for *Salmonella* spp. and 88% of these samples harbored isolates resistant to ≥1 antibiotic. Moreover, we found that 26% of antibiotic resistant *Salmonella* spp. isolates were able to transfer their resistance traits to a recipient strain of *Escherichia coli* at the rate of 1-7 x 10^{-3} per donor cells. Microbiological contamination of water was clearly evident in open water sources from northern Tanzania, and the presence of *Salmonella* spp. poses an immediate risk to anyone who consumes these waters if untreated.

Key words: Fecal bacteria, *Salmonella*, invA gene, polymerase chain reaction (PCR), water.

INTRODUCTION

When surface water is contaminated with elevated levels of fecal pollution this can pose a significant health risks for people and livestock that use these waters (Jenkins, 2008). When present *Salmonella* spp. can be transmitted and cause mild to severe enteric and systemic illnesses for example typhoid and paratyphoid (Levantesia et al.,

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2012; Yavari, 2012). Salmonellosis can be self-limiting but it can be severe in children and immunocompromised adults and the elderly (Angulo and Swerdlow, 1995). *Salmonella enterica* serovar Typhi is associated with typhoid fever in humans and is attributed to >600,000 deaths annually worldwide while other serovars do not (Zhang et al., 2003). In developing countries, illness and mortality due to water-borne salmonellosis is increasing (Atherton et al., 1995). For example, of all notifiable diseases in Tanzania, 20% were attributable to typhoid fever alone in 2012 (Mwang'onde et al., 2013).

Antibiotic resistance can exacerbate the Salmonellosis burden (Glynn et al., 1998). Antibiotic resistant bacteria can persistent in different environments due to selection pressure from antibiotic use, and co-selection of genetically linked traits (Call et al., 2008). When resistance confers little fitness cost to the bacteria, resistance can persist while socioeconomic and ecological factors contribute to dissemination of these organisms to other populations (Choffnes et al., 2010). The prevalence of resistant bacteria will be greater where antimicrobial use is more frequent (Goossens et al., 2005), particularly when people can easily access antibiotics without prescription (Plachouras et al., 2010). Commensal enteric bacteria such as some *Escherichia coli* in animals and people probably serve as reservoirs of antibiotic resistance traits (Shehabi and Odeh, 2006). These commensal bacteria have the ability to transfer resistance to other bacteria, including pathogens, through horizontal gene transfer. The ability of bacteria to acquire and disseminate resistance traits via mobile genetic elements such as plasmids has increasingly contributed to the development of multidrug-resistance in the past 50 years (Hawkey and Jones, 2009).

Transmission of coliforms and *Salmonella* spp. primarily occurs via consumption of food and water that is contaminated with fecal pollution (animal and human) (Keddy et al., 2009). *Salmonella* spp. and *E. coli* can survive in water for prolonged periods (>2 weeks) (Haznedaroglu et al., 2012) and can be found in water sources such as rivers, tanks, taps and wells that have been contaminated with human or animal feces (Yavari, 2012). For many years fecal bacteria have been used as indicators of the microbial quality of drinking water (Schriewer et al., 2010). According to World Health Organization guidelines, drinking water supplies should have no detectable *E. coli* per 100 ml samples of water (World Health Organization (WHO), 2011).

In Tanzania *Salmonella* spp, including serovars Paratyphi A, Choleraesuis and Enteritidis, were detected in clinical specimens, milk and water samples (Lubote et al., 2014; Mwang'onde et al., 2013). Importantly, there is a serious lack of potable water in some Tanzanian communities. Most of the polluted surface water resources are shared between animals (livestock and wild) and people on a day-to-day basis. The goal of this study was to ascertain the microbiological quality of select water sources in northern Tanzania and to ascertain the presence of *Salmonella* spp. and the antibiotic resistance phenotypes of recovered isolates.

METHODOLOGY

Sample collection

Water samples were collected from sites that were easily accessed including, ponds, rivers, taps, wells and lake waters from northern Tanzania between March and August, 2014. Samples were collected from Kilimanjaro (Moshi Municipal, Moshi Rural and Hai districts), Arusha (Arusha City, Arumeru, Longido and Monduli Districts), and Manyara (Simanjiro and Babati Districts) regions, after obtaining permission from the local authorities (Table 1). Water samples (100 to 500 ml) were collected in sterile containers and transported in a cooler to the lab and sample processing started within six hours after collection. In this study, we defined rivers as freshwater flowing in defined channels and these waters were typically used for irrigation and livestock watering. Streams were defined as smaller volumes of water that were diverted from the river for irrigation before returning to rivers. We defined tap water as running water supplied by tap (valves) and most of this water originated from springs, upstream river segments or boreholes. Well waters originated form of ground water accessed through drilling or digging and most of the wells in the study area were covered to protect them from contamination. Ponds were defined as open bodies of standing, shallow water created by artificial impoundments designed to capture rain runoff, while lakes were defined as more permanent surface waters that typically have some river or stream input. In the study areas the rainy season falls between March and May months. Rivers were also divided into upstream and downstream segments. Upstream areas experienced little human activity near water sources and were considered at low risk of receiving fecal contamination from human or animal sources. Downstream segments included areas with high risk of impact from fecal contamination and these locations had clear evidence of human and animal interactions.

Detection of fecal bacteria

Bacteria in water samples were detected by using membrane filtration with 47 mm acetate filters with a nominal pore size of 0.45 µm (Cellulose Nitrate filter, Sartorius Stedium Biotech GmbH, Goettingen Germany) (Standard method, 2003). In brief, for each well and tap water sample 100 ml of water was filtered directly. Water samples from rivers, ponds and streams were diluted 1:10 in distilled water before filtering. Sterile distilled water (100 ml) was used as a negative control. All filters were placed on individual M-FC agar plates (M-FC agar, Tulip diagnostics (P) LTD, Goa, India) with rosolic acid (Sigma-Aldrich, St Louis, MO) and incubated aerobically at 44.5±0.5°C for 22- 24 h. Colonies that were light blue in appearance were recorded as fecal coliforms.

Detection of *Salmonella*

Water samples were processed using the EPA Method 1200 with modifications to detect *Salmonella* (EPA, 2012). Briefly, 100 ml bottle water sample was first shaken vigorously and 10 ml of sample (Abakpa et al., 2015) was added to 10 ml of double-strength tryptic soy broth (TSB) (Hi Media Laboratories Pvt. LTD, Mumbai, India) and incubated at 37°C for 24 h to enrich for *Salmonella*. After incubation 30 µl of enriched culture was dropped onto Modified Semisolid Rappaport-Vassiliadis (MSRV, Becton

Table 1. Location of water sampling points in northern Tanzania.

Region	Source	Latitude	Longitude
Arusha	Pond	S03.42581	E036.67314
	River	S03.39126	E036.39126
	Lake	S03.22564	E036.47234
	Tap	S03.36214	E036.68803
	Tap	S03.36837	E036.85860
	River	S03.36829	E036.85867
	Tap	S03.37371	E036.57249
	Pond	S03.37867	E036.55705
	River	S03.32912	E037.15288
	River	S03.37165	E036.79287
	River	S03.37142	E036.81347
	River	S03.37312	E036.82088
	River	S03.37521	E036.83269
	River	S03.36709	S036.83416
	River	S03.36539	S036.83276
	River	S03.36624	S036.83397
	River	S03.36571	E036.83686
	River	S03.37592	E036.84126
	River	S03.37587	E036.84135
	River	S03.50669	E036.87622
	River	S03.36824	E036.89604
	River	S03.55492	E036.96145
	River	S03.44361	E036.85692
	River	S03.43629	E036.85144
	River	S03.43355	E036.85278
	River	S03.43288	E036.85313
	River	S03.39443	E036.81447
	River	S03.39311	E036.82368
	River	S03.39265	E036.82301
	River	S03.38988	E036.83421
	River	S03.39068	E036.83582
	River	S03.39054	E036.83575
	River	S03.38673	E036.83625
	River	S03.43015	E036.85353
	River	S03.37364	E036.57236
	Tap	S03.32912	E036.69996
	Tap	S02.73015	E036.69671
	Pond	S03.37163	E036.57320
	Tap	S02.67736	E036.67984
	Pond	S03.41208	E036.47110
Kilimanjaro	Tap	S03.33246	E037.36866
	Tap	S03.32947	E037.36948
	Tap	S03.32491	E037.33352
	Tap	S03.31773	E037.33358
	Tap	S03.39883	E036.79517
	Tap	S03.34644	E037.34564
	Tap	S03.39739	E036.69127
	Tap	S03.33246	E037.36866
	Tap	S03.33888	E037.33976
	Tap	S03.33383	E037.36945
	Tap	S03.33011	E037.36808
	Tap	S03.34644	E037.34564
	Tap	S03.34162	E037.34837
	River	S03.33059	E037.36587
	Tap	S03.35779	E037.33697
	Tap	S03.28249	E037.30947
	Tap	S03.34094	E037.34882
	Pond	S03.32887	E037.15231
Manyara	Well	S04.19569	E035.74680
	Well	S04.19748	E035.75071
	Well	S04.19762	E035.74953
	Well	S04.24112	E035.74322
	Well	S04.21979	E035.74407
	Pond	S04.21807	E035.75954
	Tap	S04.19666	E035.74781
	Tap	S04.25350	E035.74680
	Tap	S04.23413	E035.74407
	Tap	S03.92235	E035.80795
	Tap	S04.21915	E035.73558
	Tap	S04.19996	E035.74638
	Tap	S04.18349	E035.75299
	Tap	S04.20405	E035.76128
	Lake	S04.23421	E035.74240

Total sampling points 73, some site were sampled twice.

Dickinson and Company Sparks, MD, USA) agar plates with 2% novobiocin. The drops were allowed to dry on the agar plates for 1 h at room temperature and the plates were then incubated at 42°C for 18 h. After incubation, motile *Salmonella* were identified by the formation of pale or whitish halos around the drop. A sterile loop was used to pick the presumptive motile *Salmonella* from the periphery of the halos and these bacteria were then streaked onto xylose lysine desoxycholate (XLD, Hi Media Laboratories Pvt. LTD) plates. The XLD plates were incubated aerobically at 37°C for 24 h. From the XLD plates, distinct colonies having pink to reddish color and a black center were picked and subcultured in Luria-Bertani medium (LB, Difo™ LB Broth Lennox, Sparks, MD USA) broth (one isolate per positive sample). The pure cultures (40 µl) were aliquoted into the wells of a 96-well plate and allowed to completely desiccate in the incubator for 24 h. The 96-well plates were then shipped to Washington State University (Pullman, WA, USA) for phenotypic characterization. Upon reception, the isolates were recovered by adding 150 µl of LB broth to the desiccated cultures followed by overnight incubation at 37°C. After incubation, glycerol (15% final) was added and the plates were stored at -80°C for further analyses that included antibiotic susceptibility profiling, macro-restriction digest assays and conjugation assays.

Assessing antibiotic resistance

Antibiotic susceptibility breakpoint assays (The European Committee on Antimicrobial Susceptibility Testing, 2014) were used to estimate the prevalence of antimicrobial resistant *Salmonella*. For this assay, isolates were considered resistant if they grew on

MacConkey agar plates containing defined concentrations of antibiotics [amoxicillin, 32 µg/ml (Amx, MP Biomedicals, Illkirch, France), ampicillin, 32 µg/ml (Amp, Fisher Scientific, Fair Lawn, New Jersey), chloramphenicol, 32 µg/ml (Chlo, Sigma-Aldrich, St Louis, MO), gentamicin, 16 µg/ml (Gn, LKT Laboratories, Inc., St. Paul, MN), cephalexin, 32 µg/ml (Cn Fisher Scientific), co-trimoxazole, 16/512 µg/ml (Cot, MP Biomedicals), tetracycline, 16 µg/ml, (Tet, GTS, San Diego, CA), and trimethoprim, 8 µg/ml (TRM, MP Biomedicals)]. *E. coli* strain H4H, which was resistant to all tested antibiotics, and *E. coli* strain K-12, which was susceptible to all tested antibiotics were included in each assay as controls for the antibiotic susceptibility test. For quality control, each test was considered valid when there was no growth for *E. coli* K-12 and growth of *E. coli* H4H (Call et al,. 2010) on the agar plates containing antibiotics.

Confirmation of *Salmonella* identity using *invA* PCR

DNA templates for a PCR assay were prepared by centrifuging 2 ml of overnight *Salmonella* cultures at 12,000 g for 10 min. The supernatants were discarded and the pellets were re-suspended in 200 µl of nanopure water and boiled for 10 min in a heat block. The boiled suspensions were centrifuged briefly (5,000 x*g*, 5 min) and 2 µl of the supernatant was used as DNA template for PCR assays. The presence of a diagnostic gene, *invA*, was assessed by PCR (Rahn et al., 1992). A total volume of 15 µl reaction mix (Green PCR master mix, Life Technologies) was used to perform PCR in the thermal cycler (C1000 Touch Thermal Cycler, Bio-Rad, Hercules, CA, USA). The amplified PCR products were visualized using 1.5% agarose gel containing ethidium bromide with a DNA ladder (Gene ruler 1 Kb) as a size standard.

Pulsed-field gel electrophoresis (PFGE)

A total of 26 *inv*A positive *Salmonella* isolates were characterized using Xbal macro-restriction digests with slight modifications of the PulseNet protocol (CDC, 2013). Briefly, overnight culture was adjusted to an optical density of 1.4 using a spectrophotometer (610 nm) and 200 µl of the optical density adjusted culture was augmented with proteinase K (10 µl at 20 mg/ml). Melted SeaKem Gold agarose (FMC BioProduct, Rockville, Maine, USA) was added and gently mixed before dispensing into appropriate plug molds (Bio-Rad, Hercules, CA) for 15 m. Plugs were incubated in ES buffer (0.5 M EDTA, pH 9.0, 1% sodium lauroyl-sacrosine) with proteinase K at 54°C for 1 h and then washed 3X for 1 h in 1 M TE and 0.5 M EDTA buffer. Plugs were then treated with Xbal for 3 h in a water bath at 37°C. Restriction fragments were then resolved by electrophoresis after inserting into 1% SeaKem Gold agarose gels by using a CHEF DRIII apparatus (Bio-Rad). Gels were immersed in 0.5X Tris-borate–EDTA buffer and electrophoresis included initial switching time of 2.2 sec and a final switching time of 68.3 sec, 6 volts and 120° angle. Fragments were resolved at 14°C for 18 h. Control strain *Salmonella enterica* serovar Braenderup (ATCC BAA-664™) was included on every gel to improve accuracy of fragment size estimates. After electrophoresis the gels were stained with ethidium bromide for 20 min and destained 3X for 20 min each with deionized water. Gel images were scanned using ChemiDoc XRS Gel Photo Documentation System (Bio-Rad) and analyzed using BioNumerics software version 4.0 (Applied Maths, Sint-Martens-Latem, Belgium). Cluster analysis was performed using the Unweighted Pair Group Method using arithmetic Averages (UPGMA) with 1% tolerance and a 0.5% optimization setting based on Dice coefficients to quantify similarities.

Conjugation experiments

We used a filter-mating protocol to determine if the AMR traits were transferrable to other bacteria via conjugation. *Salmonella* isolates were mated with a plasmid-free recipient strain (*E. coli* K-12, nalidixic acid resistant, Nal^r) as described previously (Subbiah et al., 2011). Briefly, single colonies of the recipient and donor strains were grown overnight separately in LB broth at 37°C. Equal quantities (10 µl) of overnight each culture were then added on top of a nitrocellulose (~ 1 cm x 1 cm) membrane overlaid onto an LB agar plate with no antibiotics. After 24 h of incubation (37°C), the culture on the membrane was suspended in 500 µl of sterile phosphate-buffered saline (PBS, pH 7.0) and spread onto LB agar plates containing either nalidixic acid (32 µg/ml) and amoxicillin (32 µg/ml) or nalidixic acid (32 µg/ml) and tetracycline (16 µg/ml). Colonies that grew onto these antibiotic containing agar plates were considered transconjugants. The transfer efficiency was calculated by dividing the number of transconjugants by the number of donor cells. Transconjugants were screened for the respective antibiotic resistance phenotypes.

Statistical analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) program version 20 (SPSS Inc., Chicago, IL, USA). Colony counts were analyzed and reported as $\log_{10}$-transformed counts. Analysis of variance (ANOVA) and a post hoc Tukey-Kramer test were used to compare total coliforms counts from different water sources and between different river locations (down and upstream). The correlation between coliform counts and detection of *Salmonella* spp. was estimated by using a nonparametric (Spearman) correlation test.

RESULTS

Out of 100 water samples, 63% had coliform counts >100, 13% had counts between 1 and 100, and 24% were negative for coliforms (Table 2). The mean CFU was significantly higher for pond water samples compared to other water sources (ANOVA, Tukey-Kramer test, $P<0.001$) (Figure 1). CFU counts were significantly higher for the downstream segments compared with upstream segments ($P = 0.01$, Figure 2).

Twenty-six percent of tested water samples were positive for *Salmonella* spp. (isolate identity confirmed by *invA* PCR). All lakes and ponds (n = 9) were positive for *Salmonella* spp. and none of the samples from wells were positive. There was a significant direct correlation between the number of coliforms and the presence of *Salmonella* ($r=0.46$, n=100, $P=0.01$). Twenty-three out of 26 (88.5%) *Salmonella* isolates were resistant to ≥1 antibiotics, with all of these isolates being resistant to amoxicillin (Figure 3). All *Salmonella* isolates were susceptible to ciprofloxacin, gentamicin, cefotaxime and chloramphenicol. Among the resistant isolates 23% (n=6) transferred resistance traits by conjugation with an efficiency that ranged between 1×10^{-3} and 7×10^{-3}. Horizontally transferred resistance included ampicillin, amoxicillin, tetracycline and trimethoprim. Macrorestriction digests assays showed that the 26 *Salmonella* isolates that were tested represented a diverse collection of fragment sizes (Figure 4). The exception was a cluster of five identical PFGE profiles for isolates that were collected from ponds located in the

Table 2. Details of the water samples collected and the detection of fecal coliforms and *Salmonella*.

Regions	Sources (n)[a]	Coliforms range of CFU/ml, n[b]	*Salmonella* spp (number r or s)[c]
Arusha	Lake (1)	BDL[d]	1s
	Pond (7)	>1000, 7	5r
	River (43)	>101-1000,14 >1001, 29	13r, 2s
	Tap (13)	<0, 4 >1-100, 5 >101-1000, 2 >1001, 2	1s
	Well (3)	<0, 3	0
Manyara	Lake (1)	>100-1001, 1	1r
	Pond (1)	>1001, 1	1r
	Tap (8)	<0, 5 >1001, 3	2r
	Well (5)	<0, 3 >1-100, 2	0
Kilimanjaro	Pond (1)	>1001, 1	1r
	Tap (16)	<0, 11 >1-100, 4 >101-100, 1	0
	River (1)	>1001, 1	0

[a]n = number of samples; [b]n = number of samples for the given CFU/ml range; [c]n = number of isolates from each category that were resistant to ≥1 antibiotic (r) or susceptible (s); [d]BDL = below detection limit.

Monduli region.

DISCUSSION

The majority of water samples assessed in this study (76%) exceeded a zero tolerance standard for fecal coliforms in drinking water (WHO, 2011). The average CFU of coliforms was higher in water samples obtained from ponds compared to other water sources. This can be expected given that these ponds are used as water supplies for livestock, wildlife, and people. Ponds of this nature have been identified as sources for transmission of pathogens from cattle to people in Kenya (Jenkins, 2008). During the sampling effort for this project we also observed that rivers and streams were used for bathing, laundering, and some discharge of human waste (Figure 5). Moreover, fecal load was found to be higher in downstream segments compared with upstream segments, which is consistent with anthropological impacts on these water systems. Fortunately, most tap and well water samples did not harbor fecal coliforms.

Also, a statistically significant correlation was detected between the abundance of fecal coliforms and detection of *Salmonella* spp. (r=0.46, n=100, P=0.01). A similar correlation (r <0.32) was documented for surface water in central Florida (Huang et al., 2014). Consequently, coliform monitoring may be predictive for the presence of fecal pathogens such as *Salmonella* in human-impacted waters in Tanzania. This research anticipates that other disease-causing pathogens including *Shigella*, rotavirus, *Campylobacter*, pathogenic *E. coli*, *Cryptosporidium*, and

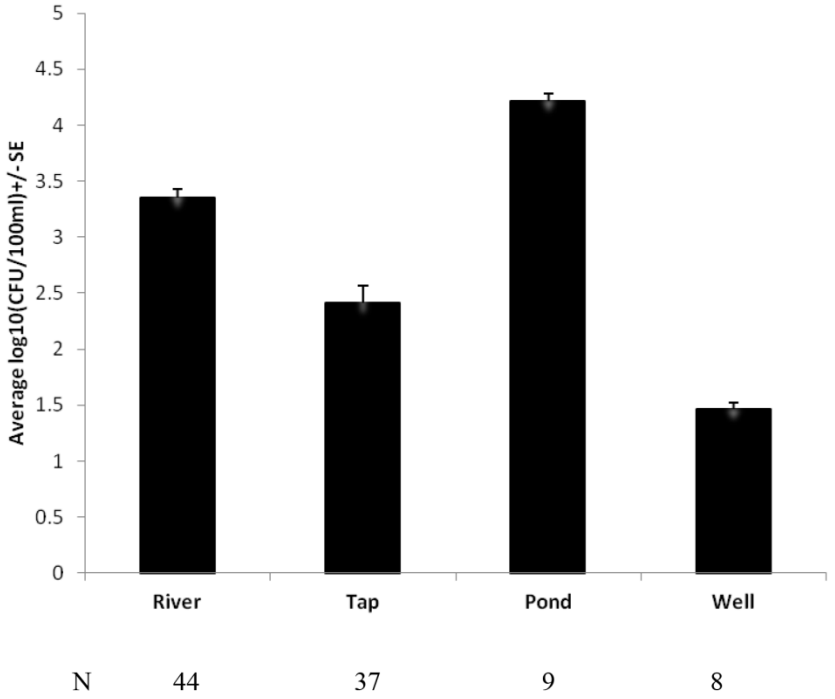

Figure 1. The average number of coliforms detected in various water sources in northern Tanzania. N: Number of samples per source. Lake was excluded from analysis as it has very less sample. Pond harbored significance higher level of fecal bacteria than other sources (ANOVA, Tukey-Kramer test, *P*<0.001).

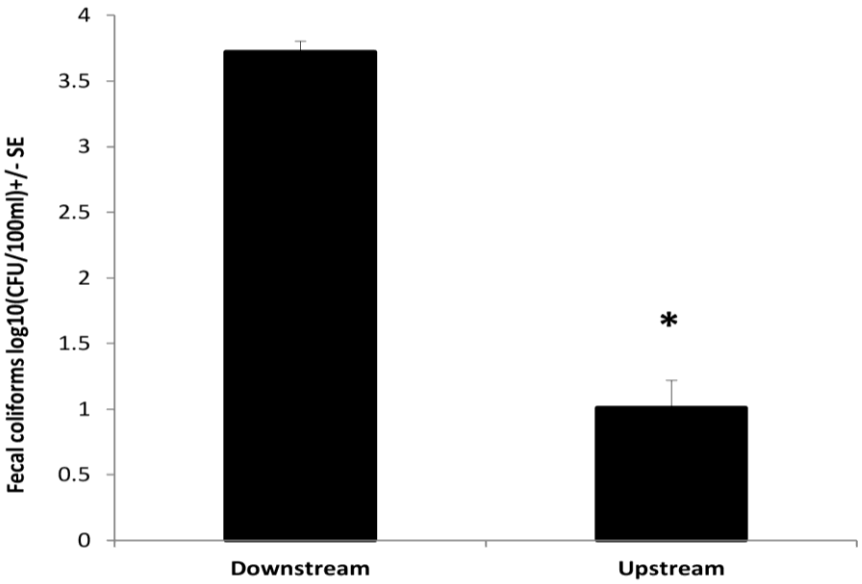

Figure 2. The average number of coliform detected both in downstream and upstream the water sources. N*: Average log10 (CFU/100ml of fecal coliform in upstream. Upstream harbour significance lower cfu compared to downstream segment (*P*=0.01).

Giardia are also associated with these waters. Thomas et al. (2013) sampled waters from urban and rural areas/ agriculture streams in Grand River watershed in Ontario, Canada and found 78.4% were positive for *Salmonella*

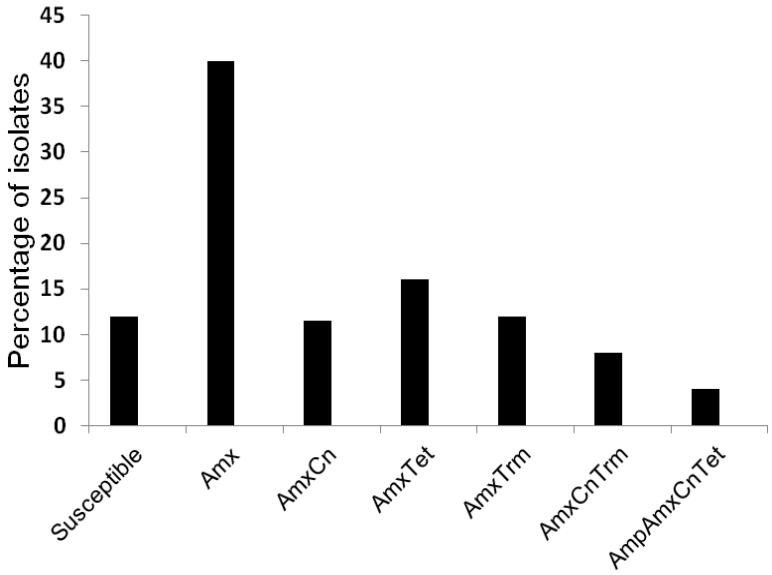

Figure 3. Antibiotic resistance phenotypes for *Salmonella* isolated from different water sources (n = 26). Amp: Ampicillin; Amx: Amoxicillin; Cn: Cefalexin; Trm: Trimethoprim: Tet: Tetracycline. No strains were resistant to ciprofloxacin, gentamicin, cefotaxime or chloramphenicol.

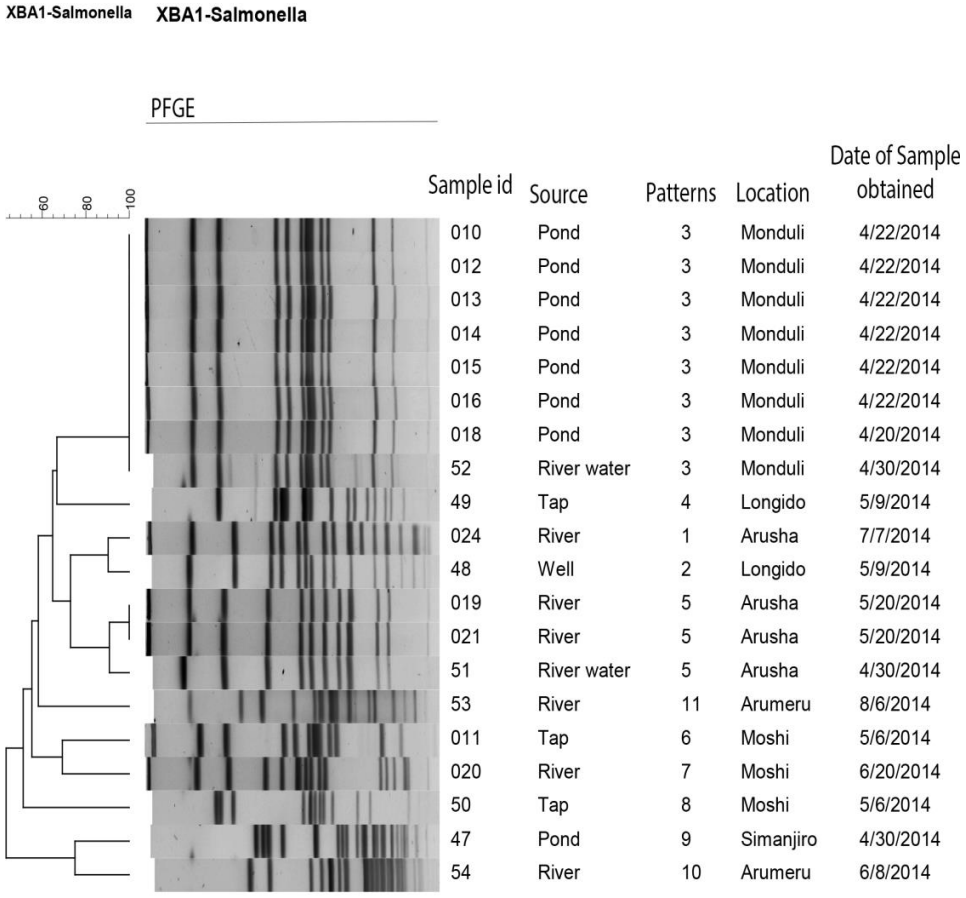

Figure 4. Dendrogram of Xbal PFGE patterns of the *Salmonella* isolates from ponds located near Monduli, Tanzania.

Figure 5. Different human activities along rivers in northern Tanzania that includes laundering.

spp. which is about three times greater than the 26% detected in our study (Thomas et al., 2013). Others have reported lower values including 12.9% from water, soils, and streams in the U.S. (Gorski et al., 2011), and 10.3% from river and irrigation water in Canada (Gannon et al., 2004). Clearly, surface water contamination from human and animal fecal waste is a recurring issue worldwide, and from a public health perspective this is particularly important in areas where people are dependent on these waters for daily needs.

All of the *Salmonella* isolates were motile and positive for *invA*, both attributes consistent with potential to cause disease (Access et al., 2015). The *invA* gene plays an essential step in the invasion of the cells of the intestinal epithelium. Amoxicillin resistance was most common (88% of *Salmonella* isolates) and amoxicillin is considered a first-line antibiotic by WHO to treat UTI infections and pneumonia (Akortha et al., 2011 and PATH, 2014). Antibiotic resistant *Salmonella* spp. were also isolated from different environmental samples including water from Nigeria, where antibiotic resistance was found to all tested antibiotics (Abakpa et al., 2015). Antibiotic-use practices (medical and veterinary) may contribute to this problem in Tanzania (Van Den Boogaard et al., 2009) and efforts to curtail unnecessary antibiotic use are likely to further reduce the overall burden of resistant strains (Carvalho et al., 2013).

In this study resistance traits ampicillin, amoxicillin, tetracycline and trimethoprim were transferred to plasmid-free bacteria via conjugation. Importantly we observed plasmid mediated horizontal gene transfer between *Salmonella* spp. and *E. coli*. Hence is likely that these resistance traits from water isolates can be transmitted back to via direct transmission to people and animals and via indirect transmission through transfer to other bacterial species and pathogens.

The macro-restriction digest assay (PFGE) showed that *Salmonella* isolates from ponds in the Monduli region are clonal. Given that these ponds serve as water supplies for a number of different households and livestock herds, it seems unlikely that they represent a discrete amplification event of a single clone as might be observed during disease outbreaks. It is more likely that the epidemiologic patterns are due to multiple fecal introductions into the environment and/or from a less genetically diverse serovars such as *S. enterica* serovar Enteritidis or Typhi (McEgan et al., 2014). Others have found clonal lineages of *Salmonella* spp. from surface water sources in Florida (McEgan et al., 2014).

If our sampling strategy is representative of other water sources in northern Tanzania, then it is likely that most waterways are polluted with fecal contamination at levels posing health risks to those consuming the water untreated. The presence of fecal coliforms is a moderate predictor for the occurrence of *Salmonella* spp., and consumption of inadequately treated water clearly puts the public at risk of disease in this area. We recommend educating people who use surface water on a daily basis to treat their water (chemical, filtration or boiling) before drinking and to find ways to protect waterways from exposure to animal and human wastes.

Conflict of Interests

The authors have not declared any conflict of interest.

ACKNOWLEDGMENTS

The authors appreciate the technical assistance provided by L. Orfe, L. Jones, D. Mshanga, S. Temba and L. Munuo. This work was funded in part by the COSTECH through the Nelson Mandela Africa Institution of Science and Technology and the Paul G. Allen School for Global Animal Health and the National Science Foundation (DEB1216040).

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