

Full Length Research Paper

Prevalence and Antibiotic Susceptibility of *Escherichia coli* and *Salmonella* spp. isolated from milk of zero grazed cows in Arusha City

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The present study assessed the antibiotic susceptibility patterns of *Escherichia coli* and *Salmonella* isolates of raw milk from zero grazed cows. A total of 65 milk samples were collected for analysis. The standard membrane filtration technique and HiCrome *E. coli* agar were used in isolation of *E. coli* from milk samples. Isolation of *Salmonella* species employed pre-enrichment in buffered peptone water followed by enrichment in Rappaport and Vassilidis broth prior to Xylose lysine deoxychocolate agar as a differential media. The isolates were analyzed for antimicrobial susceptibility to eight different types of antibiotics using disc diffusion method. The prevalence of *E. coli* was 16 (16.7%) and all the samples tested were negative for *Salmonella*. The average colony forming unit for *E. coli* was 2cfu/mL. All *E. coli* isolates tested were resistant to penicillin (100%) and amoxicillin-clavulanic acid (100%) while 15(93.8%) were sensitive to ciprofloxacin. Resistance was also observed in sulfamethoxazole-trimethoprim (43.8%), chloramphenicol (12.5%), oxytetracycline (68.8%), streptomycin (12.5%) and gentamicin (25%). Of the isolates tested, 14 (87.5%) showed multi-drug resistance pattern. These results confirm that milk from zero grazed cows in Arusha was contaminated with *E. coli*, and that most of the *E. coli* strains isolated were resistant to at least one of the antimicrobial agent commonly used in treatment of human diseases.

Key words: *Salmonella*, *Escherichia coli*, prevalence, antibiotic susceptibility.

INTRODUCTION

Milk is considered virtually sterile when secreted into the alveoli of the udder, however; thereafter it may be contaminated in the interior or exterior of the udder. While

the earlier case occurs if the animal is sick, the latter results from inappropriate handling practices and inadequate environmental hygiene and sanitation along

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the food value chain (Abate et al., 2015). Cow's milk, being nutritious with high water activity, serves as the best medium for most of microorganisms including pathogenic bacteria such as *E. coli* and *Salmonella*, that pose threats to human health (Kanyeka, 2014).

Although *Salmonella*, *Staphylococcus aureus* and *E. coli* O157:H7 are the bacteria that can be shed through milk (Ogilvie, 1986; Fagundes et al., 2012), *Coxiella burnetii*, *Listeria monocytogenes*, *Brucella* spp, *Campylobacter jejuni*, *Mycobacterium avium* subspecies *paratuberculosis*, *Bacillus cereus*, *Mycobacterium tuberculosis*, *Mycobacterium bovis* and *Yersinia enterocolitica* are the bacteria commonly contaminating milk (Dhanashekar, 2012). A study by Lubote et al., (2014), in Tanzania reported that milk quality deteriorated along the food value chain; whereby high prevalence rate of *Salmonella* and *E. coli* were found in vendors (43.8%) and 8.0×10^3 cfu/mL, shops (40%) and 6.6×10^3 cfu/mL and from producers (33.3%) and 3.0×10^3 respectively. Microorganisms isolated from animal products such as raw or unpasteurized milk and meat have long been considered as sources of human infections, where salmonellosis has been reported as one of the common food-borne infections globally (Addis et al., 2011). *Salmonella* of zoonotic origin has been reported to show increasing rates of resistance to multiple antibiotics (Mijović, 2012). Such resistance is acquired while in the host animal it is spread to humans through the food chain (Carattoli, 2003; Sisak et al., 2006; Kidie et al., 2013). Although *E. coli* is an enteric commensal bacterium in both animals and humans, pathogenic strains exist and cause different diseases including urinary tract infections, gastroenteritis, septicemia, meningitis and peritonitis (Tadesse et al., 2012).

The increasing use of antibiotics in veterinary practice is suspected to contribute to acceleration of antibiotic resistance in microorganisms found where livestock are kept (Addis et al., 2011). The irrational use of antibiotics in food producing animals could result into antibiotic residues in edible tissues and products (Darwish et al., 2013). It has been reported that, antibiotics used for treatment of human bacterial infections are used for prophylactic, therapeutic and growth promotion in animals too (Phillips et al., 2004). Bacteria that have been exposed to low doses of these antibiotics in tissues and products from these animals may be less susceptible to drugs, and when such bacteria enter the human body through consumption of contaminated foods, they may cause infections that are resistant to many antibiotics (Wang et al., 2011; Claußen et al., 2013).

A study conducted in Kilosa and Mvomero districts in Morogoro, Tanzania by Kanyeka, (2014) reported antibiotic resistance in bacteria isolated from milking containers and milk products. Example *E. coli* was reported to be resistant to amoxicillin-clavulanic acid (100%), ampicillin (100%) and amoxicillin (100%) and

Salmonella showed resistance to ampicillin (100%) and amoxicillin (100%). Lubote et al. (2014) reported that, milk may contain resistant bacterial strains as a result of cross contamination from containers, humans and the environment. Due to urbanization and limited diversity of pasture, reliance on processed commercial feed mainly cereal and oil seed by-products, zero grazed cows are prone to diseases and prominent use of antibiotics (Shem et al., 2002; Mathews Jr and Johnson, 2013). To this fact, little is known about antimicrobial resistance of bacteria that are shed by the zero grazed cows in the study area. Therefore, the present study aimed at establishing the prevalence and ascertaining the antimicrobial susceptibility pattern of *E. coli* and *Salmonella* isolated from raw milk from zero grazed cows in ten wards of Arusha city, Tanzania.

MATERIALS AND METHODS

Study site

The study was purposively conducted in ten wards (Sombetini, Baraa, Engutoto, Moshono, Moivaro, Kimandolu, Sinoni, Lemara, Daraja II and Themii) of the Arusha City where some of the residents practice dairy cattle keeping as a common economic activity (Bukuku, 2013). The Arusha City, which is the headquarter of the Arusha region is situated in the north-eastern corner of Tanzania, between latitudes 2° and 6° South and longitudes 35° and 38° East of the Greenwich (Thadeo, 2014).

Sample collection

The sample size was determined using the prevalence rate of 90% from the previous study by Lubote et al. (2014) and the formula used by Addis et al., (2011) which is;

$$N = (Z_{\alpha/2})^2 \times P(1-P) / d^2;$$

Where; N is the required sample size, Z_{α} , the normal deviation at 5% which is 1.96, P, the estimated prevalence which is 90% and d^2 , the precision of estimate considered as 0.05. According to the formula, a total of 66 samples should be used in the study. Only 65 samples were analysed for the study as one of the farmer dropped out in the last period of sample collection.

The studied households were selected randomly from the list of dairy keeping households available at the Ward Livestock Offices. From each household, milk samples were collected from only one milked cow that received medication later than others and that the withdraw period for any disease treated was over and seemed apparently health. A total of 65 milk samples each from a single cow were collected from all the teats on the udder of the selected animals. The milk samples were collected during the milking time between 17:00 and 19:00h. The udders and teats of the selected cows were washed thoroughly with warm water and then dried by using towels, then, the fore stream of milk was directed to the household milking container so as to clean the orifice hence prevent contamination by environmental bacteria. Thereafter, a stream of milk was directed to the sterile falcon tubes while avoiding the contact between the sampling container, cow's teats and the milker's hands so to prevent contamination of the samples by environmental bacteria. The milk samples were kept in a cool box at

about 4°C so as to avoid bacterial proliferation. The samples were immediately transported to the Nelson Mandela African Institution of Science and Technology (NM-AIST) laboratory for bacterial culture within five hours (Lubote et al, 2014). All media used in isolation of bacteria were from HiMedia Laboratories Pvt. LTD, Mumbai, India, were of analytical grade and used according to manufacturer's instructions.

Isolation of *E. coli*

The standard membrane filtration technique and HiCrome *E. coli* agar were used in isolation of *E. coli* from milk samples. The procedure was carried as described by Robinson and Batt (1999) and Lyimo et al. (2016). Briefly, 10ml of milk sample was diluted into 90ml of double distilled sterile water. Then, 100ml of the diluted sample was filtered through a 47mm membrane filters (cellulose nitrate filters) with pore size of 0.45µm (Sartorius Stedium Biotech GmbH, Goettingen) in a vacuum filtration system. After filtration, each filter membrane was placed on a chromogenic selective agar plate (HiCrome *E. coli* agar) and then pre-incubated at 37°C for 4h so as to resuscitate the injured or stressed bacteria, followed by incubation for 22h at 44°C. *E. coli* were picked, preserved in 15% glycerol: 85% Lysogeny broth (LB) and stored at -80°C for subsequent analysis.

Isolation of *Salmonella*

Isolation of *Salmonella* was carried out according to the procedures described by Addis et al. (2011). Briefly, 1.0ml of milk sample was pre-enriched with 9.0 ml of buffered peptone water (BPW) for 24h at 37°C. Then, 0.4ml of the non-selective pre-enrichment step was transferred to 10 ml of Rappaport and Vassilidis broth (RVS) and then incubated at 42°C for 24h. Then, a loopful (1µl) of cultured broth from the selective enrichment step were streaked onto Xylose-Lysine Deoxycholate agar (XLD) plates using a sterile wire loop and then incubated at 37°C for 24h. For samples that did not show any growth during 24h, incubation was extended to 48h.

Colony forming units

The colony forming unit per millilitre (Cfu/ml) was calculated using the formula;
 Number of colonies *dilution factor /volume plated (Baranzoni, 2014).

Antimicrobial susceptibility testing

Sensitivity toward eight different antibacterial agents (streptomycin 300 µg, penicillin 10 µg, tetracycline 10 µg, sulfamethaxazole-trimethoprim 25 µg, oxytetracycline 30 µg, gentamicin 10 µg and amoxicillin-clavulanic acid 3 µg) commonly used for disease treatment in both humans and animals, ciprofloxacin 5µg and chloramphenicol 10µg which are drugs reserved for human disease treatment was carried out. The procedure described by Lalitha (2004) was used. In brief, *E. coli* cells were resuscitated through incubation on nutrient broth (Liofilchem Bacteriology Products, Roseto) at 37°C for 24 h. The turbidity was adjusted against 0.5 Macfarland concentrations (Remel, Lenexa Kansas) by adding the *E. coli* culture into sterile normal saline 0.85% (VWR International, West Chester).

Then, sterile swab was used to spread the *E. coli* cells on the entire surface of the petri dishes that contained Tryptone soy agar (Oxoid Ltd Basingstoke, Hampshire). Antibiotic discs were aseptically placed on top of the swabbed petri dishes and the

antibiotics were allowed to diffuse at 24°C for 15 min followed by incubation at 37°C for 24 h. The zones of inhibition were measured by using a vernier calliper into the nearest millimetres in order to establish the susceptibility profile of *E. coli*. The susceptibility pattern was classified as resistant, intermediate or susceptible according to the Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals. Clin Lab Stand Inst. 2008; 28: M31–A3. Isolates that were not susceptible to one or more antibacterial agent in three or more different antibiotic classes were considered as multi-drug resistant isolates (Magiorakos et al., 2012).

Data analysis

Data obtained from the antibiotic susceptibility testing were summarized using the Microsoft Excel 2007 and presented in tabular form and bar charts. Standard deviation was also calculated using Microsoft Excel 2007.

RESULTS AND DISCUSSION

Isolation of *E. coli* and *Salmonella*

The present study aimed at assessing the prevalence and antibiotic susceptibility profile of *E. coli* and *Salmonella* isolates of zero-grazed cows' milk. Out of 65 samples, only 7(11%) were positive for *E. coli* and *Salmonella* was not detected in any of the samples tested. The highest number of *E. coli* colonies in *E. coli* positive samples was four, thus the average prevalence rate of *E. coli* in the present study was 16 (16.7%). The lowest prevalence rate of 116 (12.9%) *E. coli* has also been reported by Worku et al, (2012) in Oromia Regional State. Another study by Ekici, et al., (2004) in Turkey reported that neither *Salmonella* nor *E. coli* was isolated in all milk samples collected from individual cows while the study by Reta et al. (2016) at Jigjiga City of Somali Regional State reported a higher prevalence of 9 (30%) *E. coli* and 1 (3.3%) *Salmonella* isolates. The differences in prevalence rate of *E. coli* and *Salmonella* may be attributed to the health status of cows whose milk was sampled. In this study, milk samples were collected from apparently healthy animals and may explain for the low prevalence rates observed. The average colony forming unit for *E. coli* was 2 cfu/ml. This indicates that, *E. coli* load in all milk samples were low compared to the previous literature by Marth and Steele (2001); that cows can shed *E. coli* up to 10⁸ cfu/ml. These findings implies that, raw milk in the study area had low initial bacterial count, probably because milk samples were collected from animals that were considered apparently healthy, this has also been previously observed by Tamime (2009).

The absence of *Salmonella* in all samples is supported by the previous reports that, *Salmonella* could be shed through milk only when the animal is suffering from acute clinical salmonellosis and sometimes by carrier animals

Table 1. Summary of the diversity of *E. coli* isolates from zero grazed cow's milk.

Ward	No. of sample per ward	Number of <i>E. coli</i> positive samples per ward	Number of <i>E. coli</i> colonies per sample	<i>E. coli</i> Cfu/ml per (100 ⁻¹)	Prevalence of <i>E. coli</i> in milk/ward	Number of <i>Salmonella</i> positive samples per ward
Sombetini	6	2	1	1	33.3%	0
				1		0
Baraa	6	1	3	3	16.7%	0
Moshono	6	1	4	4	16.7%	0
Kimandolu	6	1	4	4	16.7%	0
Them	6	1	2	2	16.7%	0
Olasity	6	1	1	1	16.7%	0
Moivaro	5	0	0	0	0	0
Lemara	6	0	0	0	0	0
Engutoto	6	0	0	0	0	0
Sinoni	6	0	0	0	0	0
Daraja II	6	0	0	0	0	0
Total	65	7	16	16		0

(Wood et al., 1991; McGuirk and Peek, 2003). Furthermore, the absence of *Salmonella* in milk suggests that milk is free from bacteria in the interior of the udder only if the animal is healthy (Murphy and Boor, 2000). It has also been reported in study by Abate et al. (2015) that, milk is virtually sterile when secreted into the alveoli of the udder and, after secretion, milk may be contaminated within the udder if the animal is sick or outside the udder as a result of cross contamination. Additionally, presence of *E. coli* in milk could be due to infection of the teats by environmental *E. coli* or the milk was contaminated by *E. coli* from the environment during sampling or because of faulty laboratory procedures (Smith et al., 1985; Smith and Hogan, 1993).

Since milk samples were collected from cows that were considered apparently healthy, but had the history of medication, it could be that, *Salmonella* isolates were more sensitive whereas *E. coli* isolates might have been resistant to the administered antimicrobials. On the other hand, *Salmonella* and *E. coli* are enteric bacteria which are found in animal's intestine (Sawant et al., 2007; Ouseph et al., 2009; Tadesse et al., 2012) and their presence in milk could imply that, the animal is a carrier or infected by such bacteria (McGuirk and Peek, 2003). Table 1 shows the diversity of *E. coli* and *Salmonella* isolation from milk samples collected from ten wards of Arusha City.

Antimicrobial susceptibility testing

Among the isolates tested, 56.3% were susceptible to sulfamethoxazole-trimethoprim, chloramphenicol (37.5%), penicillin (0%), oxytetracycline (31.3%),

streptomycin (68.8%), gentamicin (12.5%), ciprofloxacin (93.8%) and amoxicillin-clavulanic acid (0%). The intermediate pattern observed were sulfamethoxazole-trimethoprim (0%), chloramphenicol (50%), penicillin (0%), oxytetracycline (0%), streptomycin (18.8%), gentamicin (62.5%), ciprofloxacin (0%) and amoxicillin-clavulanic acid (0%) and resistance pattern observed were sulfamethoxazole-trimethoprim (43.8%), chloramphenicol (12.5%), penicillin (100%), oxytetracycline (68.8%), streptomycin (12.5%), gentamicin (25%), ciprofloxacin (6.25%) and amoxicillin-clavulanic acid (100%).

Of the selected antibiotics, *E. coli* were prevalently resistant to penicillin (100%) and amoxicillin-clavulanic acid (100%). The results are similar to the findings by Idriss et al., (2014) who reported that 96% of *E. coli* isolates were resistant to amoxicillin-clavulanic in Nitra, Slovakia. Similarly, Belayneh et al., (2014) reported that, 65% of the *E. coli* isolates were resistant to penicillin in East Showa Zone of Akaki District, Ethiopia. The resistance of *E. coli* isolates to amoxicillin-clavulanic acid observed in the present study is, however, higher than the findings by Čížek et al. (2008) who reported that, 23% of the isolates were resistant to amoxicillin-clavulanic acid. The high resistance to amoxicillin-clavulanic acid and penicillin reported in the present study could be associated with the lack of professionalism in dairy farming which may contribute to misuse of these drugs. As observed in the present study, during the onsite visits, 56.9% of the dairy farmers in Arusha City kept no record of any health interventions made to their animals. Moreover, it could be due to self-medication by using experience, instructions from veterinary input shops or instructions on the label of the respective medicine, a

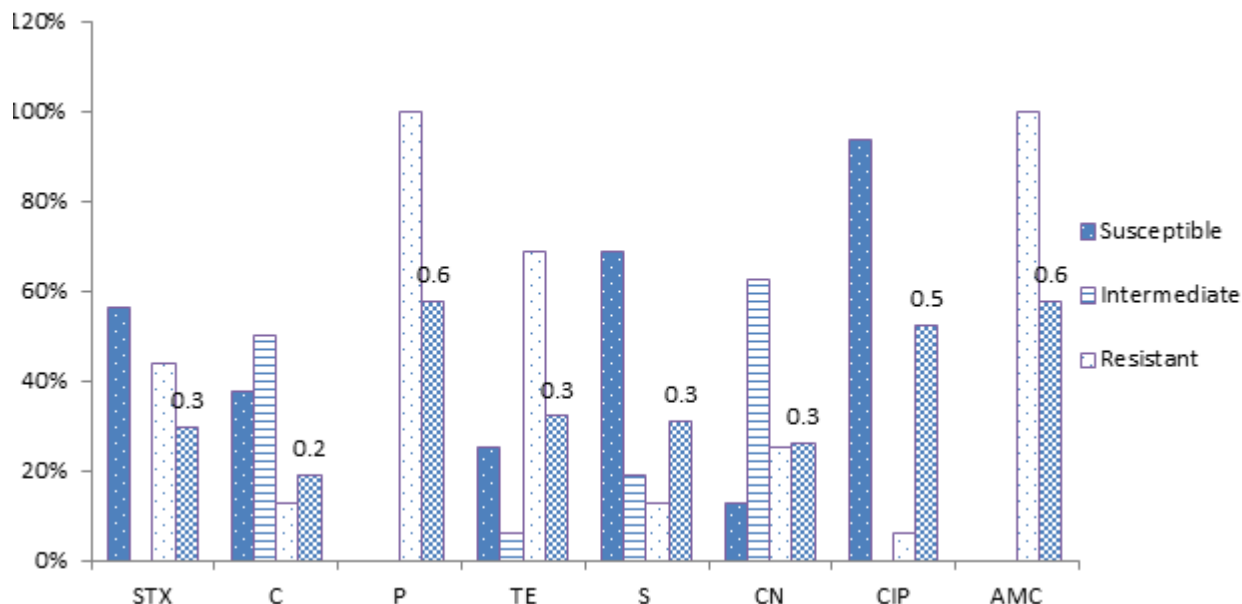


Figure 1. Susceptibility profile of *E. coli* to selected antibiotics. SXT-Sulfamethazole-trimethoprim, C-Chloramphenicol, P-Penicillin, TE-Tetracycline, S-Streptomycin, CN-Gentamicin, CIP-Ciprofloxacin, AMC-Amoxicillin-clavulanic acid.

practice that may not always result in the correct treatment of the disease. Since in Tanzania, antibiotics are sometimes sold without prescriptions (Van de boogard et al., 2011), the observed resistance could be due to increased use of antibiotics, especially the first line antibiotics which are cheap and easily accessible (Shakya et al., 2013). The susceptibility profile of *E. coli* to different antibiotics has been summarised in Figure 1 below.

The study has also revealed resistance (12.5%) and intermediate (60%) patterns to chloramphenicol. These findings are in contrast to those reported by Belayneh et al. (2014) in East Showa Zone of Akaki District, Ethiopia, who reported 100% sensitivity of *E. coli* to chloramphenicol. In this study, a lower proportion (12.5%) of the *E. coli* isolates were resistant to chloramphenicol compared to the high resistance rate (40%) reported by El-Zubeir and El-Owni (2009). The resistance to chloramphenicol reported in the present study may be associated with indiscriminate use of this abandoned antibiotic. Some studies in Tanzania and Nigeria reported that, chloramphenicol is irrationally used in animals as evidenced by the presence of its residues in poultry and poultry products (Nonga et al., 2010; Darwish et al., 2013). However, it may be due to transfer of resistant genes as a result of cross contamination between humans, animals and the environment (Bischoff et al., 2005; Salehi and Bonab, 2006) or use of other antibiotics belonging to aphenicol group (Ruzauskas et al., 2009). The susceptibility pattern of *E. coli* from all the sampling sites is summarized in the Table 2.

Of all the isolates tested, 93.8% were susceptible to ciprofloxacin. The prevalence of sensitivity to

ciprofloxacin in this study is lower compared to the results by Lehtolainen (2004) and Persson et al. (2011) who reported 100% susceptibility of the isolates to ciprofloxacin. The higher sensitivity to ciprofloxacin may imply that the drug is not being used in dairy farming to treat animal diseases. This may be attributed by the fact that, the drug is critical for human medicine and prohibited for use in food animals (Boothe et al., 2006; Pallo-Zimmerman et al., 2010).

Among the *E. coli* isolates tested, 87.5% were multi-drug resistant. Multi-drug resistance pattern of *E. coli* has also been reported by Haque (2013) in Bangladesh and by Memon et al. (2012) in Eastern China. Of the multi-drug *E. coli* resistant isolates, 50% showed multi-drug resistance to sulfamethoxazole-trimethoprim, penicillin, tetracycline and amoxicillin-clavulanic acid. The multi-drug resistance pattern observed could be the result of accumulation of resistance genes in the plasmids, each coding for resistance to a specific antibiotic and or multi-drug efflux pump each pumping out more than one antibiotic (Nikaido, 2009). Development of multi-drug resistant bacteria is a threat to public health because it leads to ineffective treatment of infections and poor recovery of the patients (Levy and Marshall, 2004; Magiorakos et al., 2012).

Almost all 16 *E. coli* isolates showed resistance to at least one antimicrobial agent tested and, more than half, 87.5% (14) showed multi-drug resistance pattern to the tested antibiotics. Although the resistant *E. coli* were isolated from samples that were not tested for antibacterial residues, there is a possibility that antibacterial residues were present in the milk as it has been reported by other workers in Tanzania (Karimuribo

Table 2. Antibacterial susceptibility profile of *E. coli* isolates of zero grazed cow's milk to selected antibiotics

WARDS	Isolate No.	Sulfamethaxazole-trimethoprim	Chloramphenicol	Penicillin	Tetracycline	Streptomycin	Gentamicin	Ciprofloxacin	Amoxicillin-clavulanic acid
Sombetini	1	-	+/-	-	-	+/-	+/-	+	-
	2	+	+	-	-	+	+	+	-
Baraa	3	+	+/-	-	-	+	+/-	+	-
	4	+	+	-	+/-	-	+/-	+	-
	5	-	+	-	-	+/-	+/-	+	-
Moshono	6	+	+/-	-	+	+	+/-	+	-
	7	+	-	-	+	+	+/-	+	-
	8	+	+	-	-	+	-	+	-
	9	+	+/-	-	-	+	-	+	-
Kimandolu	10	-	+/-	-	-	+	+/-	+	-
	11	-	-	-	-	+	-	+	-
	12	-	+/-	-	-	-	-	+	-
Themi	13	+	+/-	-	+	+	+/-	-	-
	14	+	+	-	+	+	+/-	+	-
Olasity	15	-	+/-	-	-	+/-	+	+	-
	16	-	+	-	-	+	+/-	+	-

et al., 2005; Kurwijila et al., 2006). Therefore, more extensive research is needed to establish the magnitude of the antimicrobial residues and a concomitant antimicrobial resistance in food animals. The multidrug resistance pattern of *E. coli* isolates is summarized in Table 3.

CONCLUSION AND RECOMMENDATION

A total of 16 *E. coli* isolates were isolated from 65 milk samples examined. Almost all 16 isolates showed resistance to at least one antibacterial agent tested and, more than half (14, 87.5%) showed multi-drug resistance pattern to the tested antibiotics. All 16 *E. coli* isolates were resistant to

the first line antibiotics, penicillin and amoxicillin-clavulanic acid, probably due to their frequent use in dairy units. However, some of the isolates showed resistance to chloramphenicol and ciprofloxacin drugs that are prohibited for use in food-producing animals. This may be due to either illegal use of the drugs or transfer of resistant genes as a result of interaction with human ecosystem.

Although the resistant *E. coli* were isolated from milk samples that were not tested for antimicrobial residues, there is a possibility that antimicrobial residues were present in the milk. Therefore, extensive research is proposed to establish the relationship between antimicrobial resistance and antimicrobial residues in food animals as well as

to detect the pathogenic *E. coli* from the raw cow's milk.

Furthermore, public health education should be given to the public concerning the prudent use of antibiotics so as to avoid the problem of antibiotic resistance. Additionally, legislation is required to enforce proper use of animal and human medicines to minimize cross-transmission of resistant genes from animals to humans and vice versa.

Conflict of Interests

The authors have not declared any conflict of interests.

Table 3. Proportion *E. coli* that were multi-drug resistance

Combination of drugs to which multidrug resistance was observed	Number of <i>E. coli</i> isolates that showed multidrug resistance to the combination	Percentage proportion of <i>E. coli</i> that showed multidrug resistance to the combination (%)
Sulfamethoxazole-trimethoprim, penicillin, Tetracycline and amoxicillin-clavulanic acid	7	50
Penicillin, tetracycline and amoxicillin-clavulanic acid	2	14.3
Chloramphenicol, penicillin and amoxicillin-clavulanic acid	1	7.14
Penicillin, streptomycin and amoxicillin-clavulanic acid	1	7.14
Chloramphenicol, gentamicin and amoxicillin-clavulanic acid	1	7.14
Penicillin, ciprofloxacin and amoxicillin-clavulanic acid	1	7.14
Penicillin, tetracycline, gentamicin and amoxicillin-clavulanic acid	1	7.14
Penicillin, streptomycin and amoxicillin-clavulanic acid	1	7.14

REFERENCES

- Abate M, Wolde T, Niguse A (2015). Bacteriological Quality and Safety of Raw Cow's Milk in and around Jigjiga City of Somali Region, Eastern Ethiopia. *IJRBS*. 3:48-55.
- Addis Z, Kebede N, Sisay Z, Alemayehu H, Wubetie A, Kassa T (2011). Prevalence and antimicrobial resistance of *Salmonella* isolated from lactating cows and in contact humans in dairy farms of Addis Ababa: a cross sectional study. *BMC infect. Dis.* 11(1):222.
- Baranzoni M (2014). Advances in methods to detect, isolate and quantify foodborne pathogens.
- Belayneh R, Belihu K, Tesfaye A (2014). Microbiological study on bacterial causes of bovine mastitis and its antibiotics susceptibility patterns in East Showa Zone, Akaki district, Ethiopia. *J. Vet. Med. Anim. Health*, 6(4):116-122.
- Bischoff M, White G, Hume E, Poole L, Nisbet J (2005). The chloramphenicol resistance gene *cmlA* is disseminated on transferable plasmids that confer multiple-drug resistance in swine *Escherichia coli*. *FEMS microbial. Lett.* 243(1):285-291.
- Boothe M, Boeckh A, Simpson B, Dubose K (2006). Comparison of pharmacodynamic and pharmacokinetic indices of efficacy for 5 fluoroquinolones toward pathogens of dogs and cats. *J. Vet. Intern. Med.* 20(6):1297-1306.
- Bukuku N (2013). Awareness of health risks as a result of consumption of raw milk in Arusha City and Meru District, Tanzania. Sokoine University of Agriculture.
- Carattoli A (2003). Plasmid-mediated antimicrobial resistance in *Salmonella enterica*. *Curr. Iss. Mol. Biol.* 5(4):113-122.
- Čížek A, Dolejšká M, Novotná R, Haas D, Vyskočil M (2008). Survey of Shiga toxinogenic *Escherichia coli* O157 and drug-resistant coliform bacteria from in-line milk filters on dairy farms in the Czech Republic. *J. Appl. Microbiol.* 104(3):852-860.
- Claußen M, Bahmann D, Schmidt S (2013). Detection of antibiotic residues in food-pitfalls and optimization of agar diffusion tests in comparison with commercial test kits.
- Darwish S, Eldaly A, El-abbasy T, Ikenaka Y, Nakayama S, Ishizuka M (2013). Antibiotic residues in food: the African scenario. *Jap. J. Vet. Res.* 61:S13-S22.
- Dhanashekar R, Akkinepalli S and Nellutla A (2012). Milk borne infections. An analysis of their potential effect on the milk industry. *Germes*, 2(3): 101.
- Ekici K, Bozkurt H, and Isleyici O (2004). Isolation of some pathogens from raw milk of different milch animals. *Pak. J. of Nutr.* 3(3): 161-162.
- El-zubeir E, El-owni O (2009). Antimicrobial resistance of bacteria associated with raw milk contaminated by chemical preservatives. *World J. Dairy Food Sci.* 4(1):65-69.
- Fagundes H, Corassin C, Tavolaro P, Oliveira C (2012). Milk hygienic practices and occurrence of *Staphylococcus aureus* and *Escherichia coli* O157: H7 in small-scale dairy farms in Sao Paulo, Brazil. *Afr. J. Microbiol. Res.* 6(28):5805-5808.
- Haque E (2013). Identification, molecular detection and antibiogram profile of bacteria isolated from California mastitis test positive milk samples of crossbred cows of satkhira district. *Int. J. Vet. Sci.* 1:1.
- Idriss S, Foltys V, Tančin V, Kirchnerová K, Tančinová D, Zaujec K (2014). Mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Nitra, Slovakia. *Slov. J. Anim. Sci.* 47(1):33-38.
- Kanyeka B (2014). Assessment of microbial quality of raw cow's milk and antimicrobial susceptibility of selected milk-borne bacteria in Kilosa and Mvomero Districts, Tanzania. Sokoine University of Agriculture.
- Karimuribo E, Mdegela R, Kusiluka L, Kambarage D (2005). Assessment of drug usage and antimicrobial residues in milk on smallholder farms in Morogoro, Tanzania. *Bull. Anim. Health Prod. Afr.* 53:234-241.
- Kidie H, Bae H, Lee J (2013). Prevalence and antimicrobial resistance of *Salmonella* isolated from poultry slaughterhouses in Korea. *Jap. J. Vet. Res.* 61(4):129-136.
- Kurwijila L, Omore A, Staal S, Mdoe N (2006). Investigation of the risk of exposure to antimicrobial residues present in marketed milk in Tanzania. *J. Food Prot.* 69(10):2487-2492.
- Lalitha M (2004). Manual on Antimicrobial Susceptibility Testing (Under the Auspices of Indian Association of Medical Microbiologists). Vellore, India: CMC
- Lehtolainen T (2004). *Escherichia coli* Mastitis. PhD Thesis. University of Helsinki, Helsinki, Finland.
- Levy B, Marshall B (2004). Antibacterial resistance worldwide: Causes,

- challenges and responses. *Nat. Med.* 10: S122-S129.
- Lubote R, Shahada F, Matemu A (2014). Prevalence of *Salmonella* spp. and *Escherichia coli* in raw milk value chain in Arusha, Tanzania. *Amer. J. Res. Comm.* 2(9):1-13.
- Lyimo B, Buza J, Subbiah M, Temba S, Kipasika H, Smith W, Call R (2016). IncF Plasmids are Commonly Carried by Antibiotic Resistant *Escherichia coli* Isolated from Drinking Water Sources in Northern Tanzania. *Int. J. Microb.* 2016(2016).
- Magiorakos P, Srinivasan A, Carey R, Carmeli Y, Falagas M, Giske C, Harbarth S, Hindler J, Kahlmeter G, OLsson-liljequist B (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: International expert proposal for interim standard definitions for acquired resistance. *Clin. Microb. Infect.* 18(3):268-281.
- Marth EH and Steele J (2001). *Applied dairy microbiology*, CRC Press.
- Mathews Jr K, Johnson R (2013). *Alternative beef production systems: Issues and implications*. US Department of Agriculture, Economic Research Service, LDPM-218-01.
- Mcguirk S, Peek S (2003). Salmonellosis in cattle: A review. *In: American Association of Bovine Practitioners 36th Annual Conference*.
- Memon J, Kashif J, Yaqoob M, Liping W, Yang Y, Hongjie F (2012). Molecular characterization and antimicrobial sensitivity of pathogens from sub-clinical and clinical mastitis in Eastern China. *Prevalence.* 33(2):170-174
- Mijović G (2012). Antibiotic susceptibility of *Salmonella* spp.: a comparison of two surveys with a 5 years interval: Annual Proceeding Scientific Papers. *J. Int. Med. I. Assoc. Bulgaria* 18(1):216-219.
- Murphy S and Boor K (2000). Sources and causes of high bacteria counts in raw milk: An abbreviated review. *Dairy Food Environ. Sanit.* 20: 1-4.
- Nikaido H (2009). Multidrug resistance in bacteria. *Ann. Rev. Biochem.* 78:119.
- Nonga H, Simon C, Karimuribo E, Mdegela R (2010). Assessment of antimicrobial usage and residues in commercial chicken eggs from smallholder poultry keepers in Morogoro municipality, Tanzania. *Zoon. Pub. Health.* 57(5):339-344.
- Ogilvie H (1986). The persistent isolation of *Salmonella typhimurium* from the mammary gland of a dairy cow. *The Canad. Vet. J.* 27(9):329.
- Ouseph P, Prasanthan V, Abhilash P, Udayakumar P (2009). Occurrence and distribution of some enteric bacteria along the southern coast of Kerala. *Ind. J. Mar. Sci.* 38(1): 97.
- Pallo-zimmerman M, Byron K, Graves K (2010). Fluoroquinolones: Then and now. *Compendium: Cont. Educ. Vet.* 9.
- Persson Y, Nyman A, Grönlund-andersson U (2011). Etiology and antimicrobial susceptibility of udder pathogens from cases of subclinical mastitis in dairy cows in Sweden. *Acta Vet. Scand.* 53(1):36.
- Phillips I, Casewell M, Cox T, De groot B, Friis C, Jones R, Nightingale C, Preston R, Waddell J (2004). Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. *J. Antimicrob. Chemother.* 53(1):28-52.
- Reta A, Bereda W and Alemu N (2016). Bacterial contaminations of raw cow's milk consumed at Jigjiga City of Somali Regional State, Eastern Ethiopia. *Inter. J. Food Cont.* 3(1): 1-9.
- Robinson K, Batt A (1999). *Encyclopedia of food microbiology*, Academic press, London.
- Ruzauskas M, Virgailis M, Siugzdiniene R, Suziedeliene E, Seputiene V, Daugelavicius R, Zienius D, Sengaut J, Pavilonis A (2009). Antimicrobial resistance of *Enterococcus* species isolated from livestock in Lithuania. *Vet. Arch.* 79(5): 439-449.
- Sawant A, Hegde V, Straley A, Donaldson C, Love C, Knabel J, Jayarao M (2007). Antimicrobial-resistant enteric bacteria from dairy cattle. *App. Envir. Micr.* 73(1): 156-163.
- Shakya P, Barrett P, Diwan V, Marothi Y, Shah H, Chhari N, Tamhankar J, Pathak A, Lundborg S (2013). Antibiotic resistance among *Escherichia coli* isolates from stool samples of children aged 3 to 14 years from Ujjain, India. *BMC Infectious Diseases*, 13(1): 477.
- Shem M, Masha F, Machangu R, Kambarage D, Fujihara T (2002). Bovine mastitis in Zebu and crossbred cattle under the extensive management system in Tanzania. *Asian Aust. J. Anim. Sci.* 15(5):751-756.
- Smith K, Hogan S (1993). Environmental mastitis. *The Veterinary Clinics of North America. Food Anim. Pract.* 9(3):489-498.
- Smith K, Todhunter D, Schoenberger P (1985). Symposium: Environmental effects on cow health and performance. *J. Dairy Sci.* 68:1531-1553.
- Sisak F, Havlickova H, Hradecka H, Rychlik I, Kolackova I, Karpiskova R (2006). Antibiotic resistance of *Salmonella* species isolates from pigs in the Czech Republic. *Vet. Med.* 51(5):303-310.
- Tadesse A, Zhao S, Tong E, Ayers S, Singh A, Bartholomew J, McDermott F (2012). Antimicrobial drug resistance in *Escherichia coli* from humans and food animals, United States, 1950–2002. *Emerg. Infect. Dis.* 18(5):741-9.
- Tamime AY (2009). *Dairy powders and concentrated products*. John Wiley and Sons.
- Thadeo M (2014). Economics of urban households' cooking fuel consumption in Arusha city, Tanzania. *Sokoine University of Agriculture*.
- Van den boogaard J, Semvua H, Boeree M, Aarnoutse R, Kibiki G (2011). Assessment of antibacterial sale by using the anatomic therapeutic chemical classification and defined daily dose methodology in Moshi Municipality, Northern Tanzania.
- Wang J, Macneil D, Kay F (2011). *Chemical Analysis of Antibiotic Residues in Food*, John Wiley and Sons. P 38
- Wood D, Chalmers A, Fenton A, Pritchard J, Schoonderwoerd M, Lichtenberger L (1991). Persistent shedding of *Salmonella enteritidis* from the udder of a cow. *The Canad. Vet. J.* 32(12):738.
- Worku T, Negera E, Nurfeta A and Welearegay H (2012). Microbiological quality and safety of raw milk collected from Borana pastoral community, Oromia Regional State. *Afri. J. Food Sci. Technol.* 3:213-222.