Co-colonization by antimicrobial resistant thermophilic *Campylobacter* and *Escherichia coli* in the intestines of local chicken presented for disposal at vending areas in Morogoro Municipality, Tanzania

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SUMMARY

Although carriage of multiple potential human pathogens in known reservoirs is not uncommon, investigations addressing more than one of the pathogens in these reservoir populations are rare in Tanzania. Cloaca swabs were collected from 400 scavenging local chickens (SLC) and conveyed in Bolton broth and maximum recovery diluent for isolation of thermophilic *Campylobacter* and *E. coli*, respectively. Isolation of thermophilic *Campylobacter* adopted the Capetown protocol. The isolates were identified using phenotypic tests and eventually confirmed by polymerase chain reaction. Isolation of *E. coli* was done on McConkey agar and further detection of pathogenic *E. coli* was done on sorbitol MacConkey agar. Isolates were identified by the IMViC test and the Analytical Profile Index. Both, thermophilic *Campylobacter* and *E. coli* isolates were tested for resistance against eight commonly used antimicrobial agents in veterinary and human medicine in the study area using the disc diffusion method. Thermophilic *Campylobacter* were detected in samples from 51 birds (12.75%), most of them carrying *C. jejuni*. The proportion of SLC colonized with *E. coli* was 81.0%, of which 7.4% were colonized with pathogenic *E. coli*. Co-colonization by both thermophilic *Campylobacter* and *E. coli* was detected in 9.75% of the collected samples, 28.2% of them involving pathogenic *E. coli*. All the thermophilic *Campylobacter* isolates were resistant to cephalothin; and more than half (88.2%) to amoxycillin. Resistance to macrolides and quionlones occurred at lower frequencies. A higher frequency of *E. coli* isolates (91.7%) was resistant against cephalothin. Slightly lower frequencies of *E. coli* isolates were resistant to gentamycin, amoxicillin and erythromycin. Significantly lower frequencies of resistance were noted for nalidixic acid, ciprofloxacin, norfloxacin and azithromycin. Multidrug resistant isolates were found in 31.5% and 27.8% of thermophilic *Campylobacter* and *E. coli* isolates, respectively. SLC are reservoirs of antimicrobial resistant thermophilic *Campylobacter* and *E. coli* isolates, and are thus potential sources of human infections with these organisms. The birds should be among the targets when control of human infections with these organisms focuses at reservoir level.

Keywords: *E. coli*, Scavenging local chickens, Tanzania, Thermophilic *Campylobacter*

INTRODUCTION

Unlike other birds and mammals; chicken farming is neither suppressed, challenged, frustrated nor influenced by taboos (MAFS, 2003). In Tanzania, local chickens (SLC) contribute over 94% of the chicken population (Nonga *et al.*, 2009). They are mainly raised under scavenging production system by village farmers (MAFS, 2003). In some families these birds are hosted in human dwelling units at night. Also called rural chickens, their market is gaining popularity in towns and they contribute significantly to rural families' livelihoods. Their products particularly meat and eggs are even testier (Muhairwa *et al*, 2008). Apart from serving as a source of delicacy and income, rural chicken keeping is articulated to various social-economic functions such as prestige and dignity, payment of bride price, alternative therapy, sports, presents during festivals and manure for farm and horticultural crop production (Alders *et al*, 2002).

Zoonoses, the naturally transmissible diseases between human and animals, have significant public health implication as they represent a burden to human health (Khan *et al.*, 2009; Richt and Feldmann, 2009). Some of these diseases are caused by bacterial pathogens which include thermophilic *Campylobacter* and *E. coli*. The two bacterial species have a diverse host range and are transmitted through food of animal origin. They are the main causes of diarrhoea both in animals and humans all over the world (Silva *et al*., 2011). Avian species are particularly incriminated as the most common reservoirs and sources of these organisms for human infections.

Thermophilic *Campylobacter* are known for their capacity to colonize and become part of the normal flora of human and various animal species, including birds (Hermans *et al*., 2012). Though the organisms have been detected in stool samples from a number of animal species, avian species, poultry in particular are the most common reservoirs (Wilson *et al.*, 2008; Wysok and Uradziński, 2009). The organisms have, however, also been implicated in various cases of bacterial gastroenteritis in low, middle and high income countries (Heuer *et al*., 2001). For a long time the identification of infection sources of these pathogens has been hampered by the lack of reliable detection techniques, sporadic nature of the cases they cause as well as the ubiquitous presence of the organisms in the gastrointestinal tract and feaces of many animal and bird species (Dingle *et al.*, 2001; Frost, 2001; Newell and Feamley, 2003; Rivoal *et al.*, 2005).

Escherichia coli is one of the most common facultative anaerobic species present in the gastrointestinal tract of animals and human beings (Vinayananda *et al.*, 2017). The bacterium is frequently employed in food safety as an indicator organism of bacterial contamination (Adams and Moss, 2000). Although they commonly occur as non-pathogenic commensal bacteria, evidence shows that some *E. coli* serotypes are associated with human and animal illnesses worldwide (Nataro and Kaper, 1998; Neidhart, 1996). *E. coli* O157:H7 has emerged recently as an important food-borne human pathogen responsible for the haemorrhagic colitis and haemolytic uraemic syndrome (Karmali *et al.*, 2010).

Tanzania, as it is with many other low income countries, has scarce information on bacteriological and epidemiological aspects of many important pathogens including those of zoonotic potential. This impedes strategies towards the control of diseases caused by those pathogens. The present study was thus performed to determine the occurrence of antimicrobial resistant thermophilic *Campylobacter* spp. and *E. coli* in the intestines of scavenging local chickens at vending points. It took into consideration the potential public health risk that the chickens pose through transmission of different agents through contamination of their meat during slaughter and cross-contamination of other food products during preparation. The generated data may help public health authorities in different ways including devising control programs.

MATERIALS AND METHODS

Study area and sample collection

Samples were obtained from two local chicken vending areas located at Mawenzi and Morogoro central markets. The areas were selected on convenience basis since they are assembly grounds of chicken from various areas of the region and outside. The study involved obtaining cloacal swabs using sterile moist cotton swabs which were immediately inoculated into 4 ml of Bolton broth containing *Campylobacter* selective supplement. Samples for detection of *E. coli* were placed into maximum recovery diluents. The obtained samples were transported on ice to the public health laboratory at the College of Veterinary Medicine and Biomedical Sciences (CVMBS), Sokoine University of Agriculture (SUA) for further processing.

Bacteriological culture of collected samples for isolation of thermophilic *Campylobacter* and *E. coli*

The isolation of thermophilic *Campylobacter* adopted the Cape Town protocol, with slight modification as explained by Jacob *et al.* (2011). Detailed procedures are well documented in Komba *et al.* (2014) and Komba *et al.* (2015). *Campylobacter* isolates were preliminarily identified based on phenotypic tests; and subsequently confirmed by a molecular technique (polymerase chain reaction); details are found in Komba *et al.* (2014) and Komba *et al.* (2015). For isolation of *E. coli* a loopful of faecal homogenate in MRD was inoculated onto MacConkey agar (Oxoid, Hampshire, UK) and incubated aerobically at 37°C for 24 hours. Presumptive *E. coli* colonies were sub-cultured on new MacConkey agar (Oxoid, Hampshire, UK) plates for purification. Pure colonies were subjected to biochemical tests for identification of *E. coli* using standard methods (Mcfadden, 2000). All isolates identified as *E. coli* were inoculated on sorbitol MacConkey (SMAC) agar plates and again incubated aerobically at 37°C overnight. Phenotypic characteristics of pathogenic *E. coli*, specifically the ability to ferment sorbitol on SMAC agar as earlier described (March and Ratnam, 1986) were observed.

Antimicrobial resistance testing

The isolates were tested for resistance to various commonly used antimicrobials in the study area using the disc diffusion method on Müller-Hinton agar (Luangtongkum *et al.,* 2007). Details for the method are explained in Komba *et al.* (2014) and Komba *et al.* (2015). The isolates were tested for resistance against the following antimicrobial agents; nalidixic acid (NA, 30 μg), ciprofloxacin (CIP, 5 μg), gentamycin (CN, 10 μg), cephalethin (CL, 30 μg), amoxycillin (AML, 25 μg), norfloxacin (NOR, 10 μg), erythromycin (E, 15 μg), azithromycin (AZM, 15 μg) (Oxoid Ltd, Basingstoke, UK).

Data analysis

Collected data were cleaned in Microsoft Office Excel® 2007 (Microsoft Corporation, One Microsoft Way, Redmond, 98052-7329, USA). Descriptive statistics were computed in Epi-Info version 7 (CDC Atlanta, USA) to determine the proportion of samples positive for thermophilic *Campylobacter* and *E. coli*; and the proportion of isolates resistant to antimicrobial agents.

RESULTS

Occurrence of thermophilic *Campylobacter* and *E. coli* in the collected samples

Thermophilic *Campylobacter* were detected in 51 (12.75%) out of the collected samples (400). The vast majority of the isolates (84.3%) were *C. jejuni* whereas *C. coli* accounted for the remaining proportion (15.7%). The proportion of SLC colonized with *E. coli* was 81.0%, of which 7.4% were colonized with pathogenic *E. coli*. Co-colonization by both *E. coli* and thermophilic *Campylobacter* was observed in 9.75% of the collected samples, 28.2% of them involving pathogenic *E. coli*.

Antimicrobial resistance profiles of thermophilic *Campylobacter* and *E. coli* isolates

Results on antimicrobial resistance profiles of the isolates are presented in table 1. All the thermophilic *Campylobacter* isolates were resistant to cephalothin; and more than half to amoxycillin (88.23%). Resistance to macrolides and quionlones occurred at lower frequencies. A higher frequency of *E. coli* isolates was resistant to cephalothin (91.66%). Slightly lower frequencies of *E. coli* isolates were resistant to gentamycin, amoxicillin and erythromycin. Significantly lower frequencies of resistance were observed for nalidixic acid, ciprofloxacin, norfloxacin and azithromycin. There were no defined uniform antimicrobial resistance patterns between co-occurring thermophilic *Campylobacter* and *E. coli* isolates. Multidrug resistant isolates were found in 31.5% and 27.8% of thermophilic *Campylobacter* and *E. coli* isolates, respectively.

Table 1: Antimicrobial resistance profiles of thermophilic *Campylobacter* and *E. coli* isolates obtained from dogs in Morogoro, Tanzania

Antimicrobial Agent	Antimicrobial class	Proportion of resistant isolates (%)	
		Thermophilic *Campy* (n=51)	*E. coli* (n=324)
Nalidixic acid	Quinolone	11.8	8.33
Ciprofloxacin	Quinolone	9.8	7.71
Gentamycin	Aminoglycoside	45.1	28.7
Cephalothin	Cephalosporin	100	91.66
Amoxycillin	Penicillin	88.23	36.7
Erythromycin	Macrolide	13.72	34.9
Azithromycin	Macrolide	19.6	13.0
Norfloxacin	Quinolone	15.6	14.2

Key: *Campy* = *Campylobacter*; *E. coli* = *Escherichia coli*

DISCUSSION

A previous study in the country (Chuma *et al.,* 2016) detected thermophilic *Campylobacter* in 48 (75.0%) out of a sample of 64 SLC. The reported frequency of isolation of the organisms by these authors is higher than what was observed in this study (12.75%). Similarly, a study involving free ranging ducks (Nonga and Muhairwa, 2009) reported a higher frequency (80%) of occurrence of thermophilic *Campylobacter* (n=90). The proportions of *C. jejuni* and *C. coli* reported in the two studies (Nonga and Muhairwa, 2009; Chuma *et al.,* 2016) are however comparable to those detected in the current study. In the UK, Loc Carrillo *et al.* (2007) isolated thermophilic *Campylobacter* from 86.4% of free ranging layer chickens, 47.4% being *C. jejuni* and 52.6% being *C. coli*. In the same country, Colles *et al.* (2011) reported a peak prevalence of 88.0% for thermophilic *Campylobacter* infections in free ranging broiler breeder flock. A more or less similar prevalence (18.4%) of thermophilic *Campylobacter* to the one obtained in this study was obtained among SLC in Ethiopia (Brena *et al.*, 2015). In the study all the isolates were specified as *C. jejuni.*

The avian intestines have been known to harbor zoonotic *E. coli* strains which could be transferred directly to, and cause infections in humans (Shecho *et al.*, 2017). In this study 81.0% of the sampled SLC were found to be colonized by *E. coli*. *E. coli* have been isolated from SLC in different proportions in studies conducted earlier, both in the country (Hamisi *et al.*, 2014; Rugumisa *et al.*, 2016) and elsewhere (Manishimwe *et al.*, 2017). A study conducted in Rwanda by Manishimwe and others (2017) has recently reported an isolation frequency of *E. coli* of 79.7% from a sample of 207 free ranging chickens. Amadi *et al.* (2015) recorded an *E. coli* prevalence of 98.5% (n=205) among a population of free ranging chickens in West Indies.

Poultry production is a fastest growing industry such that poultry meat has become a major and affordable source of protein worldwide (Hemen *et al.*, 2012). SLC are particularly heavily consumed in Tanzania and other developing countries leading to an increase in their demand. Co-colonization of these avian species by both *E. coli* and thermophilic *Campylobacter* was observed in 9.75% of the collected samples. This observation translates into a significant public health risk as during slaughter and processing infected birds may contaminate meat and result into multiple pathogen infections among consumers. Poultry meat is a commodity most frequently associated with outbreaks of food-borne illnesses in humans (Shecho *et al.*, 2017). A co-contamination level of 29.3% with both *Campylobacter* and *E. coli* in a sample of 184 chicken carcasses has been recorded in the US (Zhao *et al.*, 2001). Their finding suggests co-colonization of these birds by the detected organisms during their lifetime.

Antimicrobial resistance is one of the rapidly growing problems worldwide, more so in the developing countries (Isenbarger *et al.*, 2002; Hamisi *et al.,* 2014). Frequently antimicrobial resistance has been observed among bacteria strains originating from food animals, including thermophilc *Campylobacter* and *E. coli*, posing substantial public health risk of antimicrobial resistant human infections in both developed and developing countries (Isenbarger *et al*., 2002). Fecal contamination of carcasses during slaughtering can increase the probability of spreading antibiotic- resistant bacteria to people (Álvarez-Fernández *et al.*, 2013;

Dhama *et al.,* 2013). Handling and consumption of raw or undercooked contaminated poultry meat is a potential risk factor for the transmission of bacteria, including antibiotic-resistant strains (Johnson *et al.,* 2007). Although commensal bacteria do live without causing harm to hosts, it has been suggested that they may acquire or spread resistant strains to humans and livestock (Kassa *et al.,* 2007). The chemotherapeutic implication for humans, livestock and pet animals can therefore not be ignored.

In the present study all the thermophilic *Campylobacter* isolates were resistant against cephalothin. Frequencies of resistance against quinolones and macrolides among the isolates were however significantly low in this study. Resistance to cephalosporins including cephalothin, is known to be an intrinsic attribute among *Campylobacter* species (Pezzotti *et al.,* 2003). A previous study in the country reported similar low frequencies of resistance against ciprofloxacin and norfloxacin among *C. jejuni* strains isolated from ducks (Nonga and Muhairwa, 2009). The study however reported a higher frequency of resistance against erythromycin and a lower frequency against amoxicillin in comparison to those obtained in this particular study. Significantly lower frequencies of resistant thermophilic *Campylobacter* isolates, particularly to macrolides and quinolones, have been reported elsewhere (Moore *et al.,* 2005).

The vast majority of the *E. coli* isolates in this study were susceptible to ciprofloxacin, nalidixic acid, norfloxacin and azithromycin. Moderate frequencies were noted for gentamycin, amoxicillin and erythromycin. These are among the most important and widely used antimicrobial agents in veterinary and human medicine in the study area. Results for amoxicillin, ciprofloxacin (Amadi *et al.,* 2015; Rugumisa *et al.,* 2016) and gentamycin (Manishimwe *et al.,* 2017) are in support of previous studies. Contrary to the present results, Hamisi *et al.* (2014) observed higher frequencies of resistance phenotypes against penicillins and quinolones among *E. coli* isolates recovered from SLC in the Northern part of the country. Consistently Rugumisa *et al.* (2016) and Hamisi *et al.* (2014) reported high susceptibility to chloramphenicol among SLC derived *E. coli.* The two studies however differed in observations on frequencies of resistance to sulfamethoxazole, trimethoprim and tetracycline in which Hamisi *et al.* (2014) reported relatively higher values. A higher resistance (98.8%) against erythromycin and a significantly lower resistance (3.7%) against gentamicin among SLC derived *E. coli* isolates have been reported recently in Rwanda (Manishimwe et al., 2017).

Resistance against cephalothin was the highest (91.66%) among SLC derived *E. coli* isolates. A recent study (Carvalho and colleagues, 2016) has used cephalothin to recover *E. coli* strains with potential for multi-drug resistance. The authors associate occurrence of cephalothin resistance markers, with presence of markers for chloramphenicol, tetracycline and trimethoprim-sulfamethoxazole. Resistance to cephalothin represents a public health and a clinical significance because it is commonly observed in situations with no strong selective pressures (Carvalho and colleagues, 2016). Contrary to findings in this study, Amadi *et al.*, (2015) reported a significantly lower resistance against cephalothin (1%) among SLC derived *E. coli* isolates in West Indies.

Though antimicrobial resistance is associated with antimicrobial use, in Rwanda (Manishimwe *et al.,* 2017) found no statistically significant differences in frequencies of resistant *E. coli* against different antimicrobial agents between free-range poultry farms on low antimicrobial use (30.9%) and layer and broiler production farms who were on 100% antimicrobial use. In Tanzania Rugumisa *et al.* (2016) found relatively lower frequencies of resistance to several antimicrobial agents among *E. coli* isolated from SLC in comparison with isolates from intensively kept broiler chickens. The authors attributed this finding to frequency of antimicrobial use in the two systems. This type of association was not investigated as only the free ranging chicken population was involved.

The occurrence of multidrug resistant phenotypes among thermophilic *Campylobacter* and *E. coli* isolates in this study is an observation worth noting, as they may result into human infections that may be difficult to treat. Multidrug resistance among thermophilic *Campylobacter* (Komba *et al.,* 2015) and *E. coli* (Amadi *et al.,* 2015, 31%; Manishimwe *et al.,* 2017, 43.2%) isolates obtained from SLC and other hosts has been reported by several authors previously. Since antimicrobial use in production of these chickens is not common, the environment could be a potential source of antimicrobial resistant bacteria acquired during scavenging. These bacteria can eventually be transmitted to humans via contaminated poultry products, directly or as a result of cross-contamination of other products.

To conclude, SLC in the study area are a potential source of antimicrobial resistant thermophilic *Campylobacter* and *E. coli* for humans and possibly other animals. Nodes should be identified in their production chain, from farm to table, to prevent human infections with pathogens they carry in their intestines. Regular surveillance of different potential human pathogens among SLC populations is suggested.

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REFERENCES

Adams MR, Moss MO. Food microbiology (2nd Ed). Cambridge: Royal Society of Chemistry, 2000.

Alders R, dos Anjos F, Bagnol B, Fumo A, Mata B, Young M. Controlling Newcastle Disease In Village Chickens. A Training Manual. Pp 9-13, 24-37, 40, 59, 78, 84, 87 and 90, 2002.

Álvarez-Fernández E, Cancelo A, D´ıaz-Vega C, Capita R, Alonso-Calleja C. Antimicrobial resistance in *E. coli* isolates from conventionally and organically reared poultry: a comparison of agar disc diffusion and Sensi Test Gram-negative methods. *Food Control* 30: 227–234, 2013.

Amadi VA, Watson N, Onyegbule O A, Matthew-Belmar V, Avendano E , Tiwari K, Sharma R, Hariharan H. Resistance Profiles of *Escherichia coli* Recovered from Feces of Healthy Free-Range Chickens in Grenada, West Indies. *Int J Curr Microbiol, App Sci*, 4(6): 168-175, 2015.

Brena MC, Mekonnen Y, Bettridge JM, Williams NJ, Wigley P, Sisay Tessema T, Christley RM. Changing risk of environmental *Campylobacter* exposure with emerging poultry production systems in Ethiopia. *Epidemiol Infect*, 144(3): 567-575, 2016.

Carvalho AC, Barbosa AV, Arais LR, Ribeiro PF, Carneiro VC, Cerqueira AM. Resistance patterns, ESBL genes, and genetic relatedness of *Escherichia coli* from dogs and owners. *Braz J Microbiol*, 47(1): 150-158, 2016.

Chuma IS, Nonga HE, Mdegela RH, Kazwala RR. Epidemiology and RAPD-PCR typing of thermophilic campylobacters from children under five years and chickens in Morogoro Municipality, Tanzania. *BMC Infect Dis,* 16: 692, 2016.

Colles FM, McCarthy ND, Layton R, Maiden MCJ. The Prevalence of *Campylobacter* amongst a Free-Range Broiler Breeder Flock Was Primarily Affected by Flock Age. *PLoS ONE* 6(12): e22825, 2011.

Dhama K, Chakraborty S, Barathidasan R, Tiwari R, Rajagunalan S, Singh SD. *Escherichia coli*, an economically important avian pathogen, its disease manifestations, diagnosis and control, and public health significance: a review. *Res Opin Anim Vet Sci,* 3:179–194, 2013.

Dingle KE, Colles FM, Wareing DR, Ure R, Fox AJ, Bolton FE, Bootsma HJ, Willems RJ, Urwin R, Maiden MC. Multilocus sequence typing system for *Campylobacter jejuni*. *J Clin Microbiol,* 39(1):14-23, 2001.

Frost JA. Current epidemiological issues in human campylobacteriosis. *J Clin Microbiol,* 90(S6): 85S–95, 2001

Hamisi Z, Tuntufye H, Shahada F. Antimicrobial resistance phenotypes of *E. coli* isolated from tropical free frange chickens. *IJSR,* 3(9): 34-37, 2014.

Hemen JT, Johnson JT, Ambo EE, Ekam VS, Odey MO, Fila WA. Multi-antibiotic resistance of some gram negative bacterial isolates from poultry litters of selected farms in Benue State. *IJST*, 2(8): 543–547, 2012

Hermans D, Pasmans F, Messens W, Martel A, Van Immerseel F, Rasschaert G, Heyndrickx M, Van Deun K, Haesebrouck F. Poultry as a host for the zoonotic pathogen *Campylobacter jejuni*. *Vector Borne Zoonotic Dis,* 12(2): 89-98, 2012.

Heuer OE, Pedersen K, Andersen JS, Madsen M. Prevalence and antimicrobial susceptibility of thermophilic *Campylobacter* in organic and conventional broiler flocks. *Letters App Microb,* 33: 269-274, 2001.

Isenbarger DW, Hoge CW, Srijan A, Pitarangsi C, Vithayasai N, Bodhidatta L, Hickey KW, Cam PD. Comparative antibiotic resistance of diarrheal pathogens from Vietnam and Thailand, 1996-1999. *Emerg Infect Dis,* 8(2): 175–180, 2002.

Jacob P, Mdegela RH, Nonga HE. Comparison of Cape Town and Skirrow's *Campylobacter* isolation protocols in humans and broilers in Morogoro, Tanzania. *Trop Anim Health Prod,* 43(5): 1007-1013, 2011.

Johnson JR, Sannes MR, Croy C, Johnston B, Clabots C, Kuskowski MA, Bender J., Smith KE, Winokur PL, Belongia EA. Antimicrobial drug-resistant Escherichia coli from humans and poultry products, Minnesota and Wisconsin, 2002–2004. *Emerg Infect Dis*, 13: 838–846, 2007.

Karmali MA, Gannon V, Sargeant JM. Verocytotoxinproducing *Escherichia coli* (VTEC)," *Vet Microbiol,* 140(3-4): 360–370, 2010.

Kassa T, Gebre-Selassie S, Asrat D. Antimicrobial susceptibility patterns of thermotolerant *Campylobacter* strains isolated from food animals in Ethiopia," *Vet Microbiol,* 119(1): 82–87, 2007.

Khan RE, Clouser DF, Richt JA. Emerging infections: a tribute to the one medicine, one health concept. *Zoonoses Public Health,* 56(6-7): 407-428, 2009.

Komba EVG, Mdegela RH, Msoffe PLM, Matowo, DE, Maro MJ. Occurrence, species distribution and antimicrobial resistance of thermophilic isolates from farm and laboratory animals in Morogoro, Tanzania. *Vet World,* 7(8): 559-565, 2014.

Komba EVG, Mdegela RH, Msoffe PLM, Nielsen LN, Ingmer H. Prevalence, antimicrobial resistance and risk factors for thermophilic *Campylobacter* infections in symptomatic and asymptomatic humans in Tanzania. *Zoonoses Public Health*, 62(7): 557-568, 2015.

Luangtongkum T, Morishita TY, El-Tayeb AB, Ison AJ, Zhang Q. Comparison of antimicrobial susceptibility testing of *Campylobacter* spp. by the agar dilution and the agar disk diffusion methods. *J Clin Microbiol,* 45(2):590-594, 2007.

Manishimwe R, Buhire M, Uyisunze A, Turikumwenayo JB, Tukei M. Characterization of antibiotic resistant *Escherichia coli* in different poultry farming systems in the Eastern Province and Kigali City of Rwanda. *Rev Elev Med Vet Pays Trop,* 70 (1): 13-19, 2017.

March SB, Ratnam S. Sorbitol-MacConkey mediumfor detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. *J Clin Microbiol*, 23(5): 869–872, 1986.

Mcfadden JF. *Biochemical Tests for Identification of Medical Bacteria*, Lippicott Williams and Wilkins, Philadelphia, Pa, USA, 3rd edition, 2000.

Ministry of Agriculture and Food Security (MAFS). Poultry Production Training Manual. Pp 3-4, 77, 161, 169, 201, 225-231, 273-275, 2003.

Moore JE, Corcoran D, Dooley JS, Fanning S, Lucey B, Matsuda M, McDowell DA, Megraud F, Millar BC, O'Mahony R, O'Riordan L, O'Rourke M, Rao JR, Rooney PJ, Sails A, Whyte P (2005) *Campylobacter*. Veterinary Research 36: 351-382.

Muhairwa AP, Msoffe PL, Mtambo MMA, Ashimogo G. Misingi ya ufugaji kuku wa asili. Mwongozo wa ufugaji bora wa kuku wa asili vijijini. Pp 1-36, 2008.

Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. *Clin Microbiol Rev.* 11:142–201, 1998.

Newell DG, Fearnley C. Sources of *Campylobacter* colonization in broiler chickens. *Appl Environ Microbiol,* 69(8): 4343-4351, 2003.

Nkogwe C, Raletobana J, Stewart-Johnson A, Suepaul S, Adesiyun A. Frequency of detection of *Escherichia coli*, *Salmonella* spp., and *Campylobacter* spp. in the faeces of wild rats (Rattus spp.) in Trinidad and Tobago. *Vet Med Int,* 2011: 686923, 2011.

Nonga HE, Muhairwa AP. Prevalence and antibiotic susceptibility of thermophilic *Campylobacter* isolates from free range domestic duck (*Cairina moschata*) in Morogoro Municipality, Tanzania. *Trop Anim Health Prod,* 42(2):165-172, 2009.

Nonga H, Mariki M, Karimuribo E, Mdegela R. Assessment of Antimicrobial Residues in Broiler Chickens in Morogoro Municipality, Tanzania. *PJN,* 8(3): 203-207, 2009.

Pezzotti G, Serafin A, Luzzi I, Mioni R, Perin R. Occurrence and resistance to antibiotics of *Campylobacter jejuni* and *Campylobacter coli* in animals and meat in northeastern Italy. *Int J Food Microbiol,* 82: 281-287, 2003.

Richt JA, Feldmann H. Emerging zoonoses: recent advances and future challenges. *Zoonoses Public Health,* 56(6-7): 257, 2009.

Rivoal K, Ragimbeau C, Salvat G, Colin P, Ermel G. Genomic diversity of *Campylobacter coli* and *Campylobacter jejuni* isolates recovered from free-range broiler farms and comparison with isolates of various origins. *Appl. Environ. Microbiol,* 71**:** 6216–6227**,** 2005.

Rugumisa BT, Call DR, Mwanyika GO, Mrutu RI, Luanda CM, Lyimo BM, Subbiah M, Buza JJ. Prevalence of Antibiotic-Resistant Fecal Escherichia coli Isolates from Penned Broiler and Scavenging Local Chickens in Arusha, Tanzania. *J Food Prot,* 79(8):1424-1429, 2016.

Shecho M, Thomas N, Kemal J, Muktar Y. Cloacael Carriage and Multidrug Resistance *Escherichia coli* O157:H7 from Poultry Farms, Eastern Ethiopia. *J Vet Med,* doi: 10.1155/2017/8264583, 2017.

Silva J, Leite D, Fernandes M, Mena C, Gibbs PA, Teixeira P. *Campylobacter*s spp. as a food-borne pathogen: a review. *Front Microbiol,* 2: 200, 2011.

Vinayananda CO, Fairoze N, Madhavaprasad CB, Byregowda SM, Nagaraj C, Bagalkot P, Karabasanavar N. Studies on occurrence, characterization and decontamination of emerging pathogenic Escherichia coli (STEC, ETEC and

EIEC) in table eggs. *Br Poult Sci,* doi: 10.1080/00071668.2017.1373387, 2017.

Wilson DJ, Gabriel E, Leatherbarrow AJ, Cheesbrough J, Gee S, Bolton E, Fox A, Fearnhead P, Hart CA, Diggle PJ. Tracing the source of campylobacteriosis. *PLoS Genet.* 4(9):e1000203.

Wysok B, Uradziński J. *Campylobacter* spp.—a significant microbiological hazard in food. I. Characteristics of *Campylobacter* species, infection source, epidemiology. *Pol J Vet Sci,* 12(1): 141–148, 2009.

Zhao C, Ge B, De Villena J, Sudler R, Yeh E, Zhao S, White DG, Wagner D, Meng J. Prevalence of Campylobacter spp., Escherichia coli, and Salmonella serovars in retail chicken, turkey, pork, and beef from the Greater Washington, D.C., area. *Appl Environ Microbiol,* 67(12): 5431-36, 2001.