Antimicrobial resistance profiles and pathogenic potential of *Escherichia coli* isolated from household dog faeces in Morogoro, Tanzania

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SUMMARY

Although *Escherichia coli* are known to be commensal bacteria, they have been associated with enteric and extra-intestinal illnesses in both humans and animals. Between July 2016 and February 2017, a cross sectional epidemiological study was conducted to determine the occurrence and pathogenic potential of antimicrobial resistant *E. coli* in the faeces of dogs in Morogoro Municipality, Tanzania. Fecal samples were collected from 404 household dogs in different locations of the Municipality and subjected to culture techniques for isolation of *E. coli*. *E. coli* isolates were identified by biochemical tests. Sorbitol MacConkey was used for detection of potentially pathogenic *E. coli*. Confirmed isolates were tested for resistance against eight antimicrobial agents commonly used in treatment of human and animal diseases in Tanzania. Out of the sampled dogs, 73.8% were infected with *E. coli*. Non-sorbitol fermenting *E. coli*, a feature of pathogenic strains, constituted 6.04% of all isolates (n=298). The frequency of infection was significantly higher in young dogs as compared to adults. Dog sex, species and health status had no influence on positivity to *E. coli*. All the isolates were susceptible to gentamycin, norflaxacin and ciproflaxacin; but resistant to erythromycin and cephalothin. Proportions of resistant isolates to other antimicrobial agents were 16.7%, 16.7% and 91.7% for azithromycin, nalidixic acid and amoxicillin respectively. In conclusion, household dogs in the study area are reservoirs of antimicrobial resistant *E. coli* including potentially pathogenic strains; and are therefore a potential source of human infections with these organisms. Contacts with these companion animals should involve care, particularly when young children are involved.
Keywords: Companion animals, *Escherichia coli*, sorbitol MacConkey, Tanzania

INTRODUCTION

Escherichia coli are the most widely distributed bacteria causing commensal gastrointestinal tract infections in both animals and humans (Odwar *et al.,* 2014). The organisms have however been implicated in symptomatic animal and human infectious diseases, and therefore considered as important zoonotic agents (Odwar *et al.,* 2014). The organisms are frequently used as indicator organisms in studies that aim at establishing antimicrobial resistance profiles of enteric bacteria in animal and human populations (Álvarez-Fernández *et al.,* 2013). Studies indicate that several mammals carry commensal *E. coli* in their gastrointestinal tracts where exposure to antimicrobial agents can select for resistance. Antimicrobial resistant *E. coli* may

horizontally transfer resistance traits to infectious pathogens (Levy and Marshall, 2004; Sato *et al.,* 2005; Johnson *et al.,* 2006; Belanger *et al.,* 2011; Jafari *et al.,* 2012; Moriel *et al.,* 2012). This may exacerbate the emerging problem of antimicrobial resistance among infectious agents as limited therapeutic options will be available in case of associated infections.

Overtime dogs (*Canis familiaris*), which are among the most popular companion animals, have made strong bonds with human beings especially in developed countries. In these bonds, the dogs seem to show more proximity-seeking behavior (Schoeberl *et al.,* 2012). Although previous research suggests that the attachment bonds that dogs form to their owners vary in their strengths (Rehn and Keeling, 2016), they often facilitate contacts between the two species, some of which are direct. Close contacts between humans and companion animals including dogs may allow interspecies transmission of zoonoses, including antimicrobial resistant bacteria (Guardabassi *et al.,* 2004).

The role of dogs as reservoirs of human infections has been determined for a number of pathogens elsewhere. Limited data are available in Tanzania regarding the potential of dogs to serve as reservoirs of pathogens capable of causing disease in man. Therefore this study was carried out in order to establish the antimicrobial resistant profiles and pathogenic potential of *E. coli* isolates from dogs. The results obtained in this study may help public health professionals in their efforts to control pet borne zoonoses.

MATERIALS AND METHODS

Study area

This cross sectional study was conducted in Morogoro Municipality which is located at latitude 5.7-10°S and longitude 35.6-39.5°E; with an elevation of 1200 m above sea level. The annual average rainfall ranges between 500 and 1800 mm; and the temperature range is between 18°C - 28°C. Fecal samples were collected from dogs in different households around Morogoro Municipality. Specifically sampled dogs were reached out in the following areas; Kihonda, Mazimbu, Forestry, Mzumbe and SUA. Samples were also collected from dogs that were sent for dipping and treatment at the animal hospital of College of Veterinary Medicine and Biomedical Sciences (CVMBS) of Sokoine University of Agriculture. Laboratory analysis of the collected fecal samples was done at the College.

Sample size estimation and sample collection

The sample size for this study was estimated by using the formula developed previously (Thrusfield, 2005) i.e. $N = Z^2P(1-P)/d^2$ where N is the sample size, Z is a multiplier from the normal distribution i.e. 1.96, P is the expected prevalence (50% used since the prevalence is not known), d is the precision level set at 0.05 (95% confidence interval). Therefore the sample size was 385 although slightly higher number of dogs (404) was sampled in the study. Fresh feces were collected from the study dogs into sterile containers containing maximum recovery diluent (MRD) and conveyed on ice to the Microbiology laboratory at CVMBS.

Isolation of *E. coli* from collected faecal samples

In the laboratory a loopful of fecal homogenate in MRD was inoculated onto MacConkey agar (Oxoid Ltd, Basingstoke,

UK) and incubated at 37°C for 24 hours. Presumptive *E. coli* colonies were sub-cultured on new MacConkey agar (Oxoid, Hampshire, UK) plates for purification. Pure cultures were inoculated onto sorbitol MacConkey (SMAC) agar (Oxoid Ltd, Basingstoke, UK) and incubated overnight at 37°C to identify pathogenic *E. coli* which appear pale/colourless.

Identification *E. coli* isolates

The preliminary identification of *E. coli* isolates relied on colonial characteristics and biochemical tests (Indole Test, Methyl red test, Voges-Proskauer, Citrate; IMViC). Pure colonies of *E. coli* isolates that had inconclusive results on Indole test and those suspected to be pathogenic (non-sorbitol fermenters) were cultured on brilliance *E. coli*/coliform selective agar and incubated overnight at 37°C. On this medium the colonies of *E. coli* appear bluish purple in colour. Confirmation of pathogenic *E. coli* isolates adopted a method described earlier (Hamisi *et al.*, 2014; Shimizu *et al.*, 2017). Confirmed isolates were subjected to antimicrobial susceptibility testing.

Antimicrobial resistance testing of the isolates

Antimicrobial resistance testing of *E. coli* isolates was performed by disc diffusion method. Briefly, bacterial suspensions were prepared in sterile normal saline and adjusted to a turbidity equivalent to a 0.5 McFarland standard. The suspensions were then spread onto well dried Mueller-Hinton agar (Oxoid Ltd, Basingstoke, UK) plates over which antimicrobial discs were dispensed. The plates were then incubated at 37°C for 24 hours followed by measuring the diameters of inhibition zones. Interpretation of results was guided by both standardized tables supplied by the National Committee on Clinical Laboratory Standards (currently known as Clinical and Laboratory Standards Institute) (NCCLS, 2002) and manufacturer's instructions. The isolates were tested for resistance against nalidixic acid (NA, 30 µg), ciprofloxacin (CIP, 5 µg), gentamycin (CN, 10 µg), cephalothin (CL, 30 µg), amoxycillin (AML, 25 µg), norfloxacin (NOR, 10 µg), erythromycin (E, 15 µg), azithromycin (AZM, 15 µg) (Oxoid Ltd, Basingstoke, UK). Multi-drug resistance was considered when an isolate was resistant to more than two classes of antimicrobial agents.

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 dogs positive for pathogenic *E. coli* in the study population and the proportion of isolates resistant to antimicrobial agents.

RESULTS

Occurrence of *E. coli* among the sampled dogs

In total 404 dogs were screened for the occurrence *E. coli* in their gastrointestinal tracts. The distribution of the dogs by location was as follows: 96 from Kihonda, 81 from Mazimbu, 83 from Forestry, 65 from Mzumbe, 30 from SUA and 49 were those brought for dipping and treatment at CVMBS Hospital. A large number of sampled dogs (73.8%) were adult and the remaining (26.2%) were puppies. The proportions of male dogs (52.5%) and female dogs (47.5%) were more or less similar. Eighty percent of the dogs were of

mixed breed and the remaining proportion (20.3%) constituted German shepherd. From the study population of 404 dogs, 298 (73.76%) were infected with *E. coli*, out of which 6.04% were found to be positive for potentially pathogenic *E. coli*. The frequency of infection was significantly higher in young dogs as compared to adults. There was no influence of sex, dog species, location and health status of the dog on infection with *E. coli*.

Table 3. Frequencies of occurrence of *E. coli* in different categories of dogs in Morogoro Municipality, Tanzania

Attribute	Category (n)	Proportion of dogs positive for *E. coli* (%)	P value
Sex	Male (212)	7.54	0.138
	Female (192)	4.16	
Breed	Mongrel (323)	4.90	0.090
	German shepherd (n=81)	9.70	
Age	Adult (298)	4.00	0.004
	Puppy (106)	11.33	
Health status	Ill health (60)	10.00	0.136
	Apparently health (n=344)	5.23	

Antimicrobial resistance profiles among isolated *E. coli* from dogs

Around 97.3% of the *E. coli* isolates obtained in this study were resistant to at least one antimicrobial agent. Lower frequencies of resistance were noted for gentamycin, norflaxacin and ciprofloxacin. Frequencies of resistant isolates against the different antimicrobial agents are as shown in Table 2. Resistance to at least three classes of antimicrobial agents was observed in 23.5% of all the isolates.

Table 2. Antimicrobial resistance profiles of *E. coli* isolates from dogs in Morogoro, Tanzania

Antimicrobial agent		Proportion of resistant isolates (%)
Name	Class	
Erythromycin	Macrolide	100
Gentamycin	Macrolide	12.8
Azithromycin	Macrolide	16.7
Norfloxacin	Quinolone	10.7
Ciproflaxacin	Quinolone	6.7
Cephalothin	Cephalosporin	100
Nalidixic Acid	Quinolone	16.7
Amoxycillin	Penicillin	72.2

DISCUSSION

This study reports occurrence of *E. coli* at a frequency of 73.76% in a sample of 404 dogs. Out of the obtained isolates 6.04% were identified as potentially pathogenic *E. coli.* Elsewhere in Turkey 94 (22%) *E. coli* isolates were obtained from 428 dog faecal samples (Aslantaş and Yilmaz, 2017), out of which 34 (36.2%) were pathogenic. The proportion of pathogenic *E. coli* among dog isolates reported by these authors was significantly higher than the proportion that the current study reports. A more or less comparable proportion of pathogenic *E. coli* (3.6%) to the one obtained in the current study was however observed by Jay-Russell *et al.* (2014) at the United States-Mexico Border in a study that involved collection of faecal samples from 358 domestic dogs.

The SMAC agar, a variant of conventional MacConkey agar (Novicki *et al.*, 2000), was adopted in this study for characterization of *E. coli* isolates into

pathogenic serotypes. Conventionally the medium has been employed in the detection *E. coli* serotype 0157:H7, which is frequently involved in hemorrhagic colitis causing bloody diarrhoea in human beings (March and Ratnam, 1986). On this medium, *E. coli* 0157:H7 does not ferment sorbitol, unlike most *E. coli* strains (March and Ratnam, 1986). According to previous researchers (March and Ratnam, 1986), SMAC medium had a sensitivity of 100% in the detection of *E. coli* 0157:H7. The authors recommended regular use of SMAC medium especially for culturing bloody stools (March and Ratnam, 1986); as they consider it to be a simple, inexpensive, rapid, and reliable means of detecting *E. coli* 0157:H7. In Trinidad and Tobago, however, researchers found that all the 19 NSF *E. coli* isolates from wild rats were negative for the O157 strain (Nkogwe *et al.,* 2011). This implies that not all NSF *E. coli* isolates are O157. Nevertheless, it has been revealed that diarrheagenic *E. coli* (DEC) of non-O157:H7 serotypes are more responsible for extreme human infections, and their clinical significance is considered to be great (Majalija *et al.,* 2008).

Young dogs were frequently infected with *E. coli* as compared to adult dogs. Most of these were brought to the University animal teaching hospital for veterinary care. This finding of comparatively higher infection rate with *E. coli* in young dogs than adult dogs contradicts an observation made earlier (Jay-Russell *et al.,* 2014). The authors reported comparable frequencies of infection between young and adult dogs with *E. coli.* In line with the current study, however, the authors observed no influence of sex, dog species and health status of the dog on infection with *E . coli*.

Apart from food animals, household pets are also regarded as natural reservoirs of antimicrobial resistant (AMR) bacteria, including those with potential to cause infections in humans (Johnson *et al.,* 2001; Shaheen *et al.,* 2010; Chung *et al.,* 2017). Antimicrobial resistant *E. coli* isolates have been detected in both apparently healthy household dogs (Carattoli *et al.,* 2005; Sun *et al.,* 2010; Wedley *et al.,* 2011; Franiek *et al.,* 2012; Tamang *et al.,* 2012; Sallem *et al.,* 2013; Gandolfi-Decristophoris *et al.* 2013; Wedley *et al*., 2017) and hospitalised dog populations (So *et al.,* 2012; Tuerena *et al.,* 2016). All the *E. coli* isolates detected in dog faeces in the present study were resistant to erythromycin and cephalothin. Recently Carvalho and colleagues (2016) used cephalothin to recover *E. coli* strains that exhibited potential resistance to multiple antimicrobials. The authors point out that resistance to cephalothin is of clinical significance because it is commonly observed in situations with no strong selectivity. They further disclose the association between cephalothin resistance markers, with markers for chloramphenicol, tetracycline and trimethoprim-sulfamethoxazole. High frequencies of resistance among *E. coli* isolates against erythromycin have been reported by others (Smet *et al.,* 2008; Poeta *et al.,* 2005, 2006; Ossiprandi *et al.,* 2008; Jackson *et al.,* 2009). Elsewhere erythromycin is used for the treatment of dogs and cats in a range of infections including those of the urinary tract and upper respiratory tract (Guardabassi *et al.,* 2004). Some authors (Jackson *et al.,* 2009) mention the frequent use of these antimicrobial agents as a cause of selective pressure for antimicrobial resistance.

Levels of resistance in the current study were lower for azithromycin, nalidixic acid, norfloxacin and ciprofloxacin. The finding indicates that the two classes of

antimicrobial agents (macrolides and quinolones) could be successfully used in treatment of *E. coli* infections in the study area. A similar low level of resistance against nalidixic acid among *E. coli* isolates has been reported recently (Wedley *et al.,* 2017).

This study observed multidrug resistance in 23.5% of the *E. coli* isolates from dogs. The occurrence of multi-drug resistance among *E. coli* isolates is an observation that has been reported in several studies involving both humans and animals (Sáenz *et al.,* 2001; Ahmed *et al.,* 2015; Carvalho *et al.,* 2016). A study by Paula and Marin (2008) reported multidrug resistance phenotypes among all *E. coli* isolates (n=92) obtained from 25 diarrheic dogs. Carvalho and others (2016) observed multidrug resistance among all *E. coli* isolated from both dogs and their owners. Johnson *et al.* (2006) reported multidrug-resistant *E. coli* transmission from a dog to its owner. A report is also available that indicates *E. coli* with similar antimicrobial resistance patterns and genes in humans and dogs sharing a household (Johnson and Clabots, 2006). In UK 561 *E. coli* isolates were obtained from faecal samples collected from 580 dogs, out of which 260 (46.35%) were AMR. Of the AMR *E. coli* isolates 106 (40.77%) were identified to be MDR (Wedley *et al*., 2017). Considering their close relationship and frequent contact with humans, this finding of occurrence of MDR *E. coli* in dog faeces may represent a reason for concern as they may be a potential reservoir of AMR bacteria or resistance determinants for human beings (Guardabassi *et al.,* 2004; Boerlin and White, 2006; Paula and Marin, 2008; Jackson *et al.,* 2009; Wedley *et al*., 2017).

Researchers have linked antimicrobial resistance to poor animal welfare as it results into limited choices of drugs for treatment of infections thereby increasing morbidity and mortality rates (Tuerena *et al.,* 2016; Wedley *et al.,* 2017). The phenomenon elevates the treatment cost of both humans and animals and so increases financial burden (Dallap Schaer *et al.,* 2010; Baker *et al.,* 2012; Smith and Coast, 2013; Tansarli *et al.,* 2013). Direct contact between these companion animals and humans through their close relationships, creates favorable conditions for interspecies pathogen transmission (Guardabassi *et al.,* 2004; Aslantaş and Yilmaz, 2017; Chung *et al.,* 2017) including AMR bacteria species. The risk is higher in children as they have closer physical contacts with dogs as well as with contaminated households.

In summary, a significant number of dogs involved in this study carried AMR *E. coli*, including those with pathogenic potential. This has serious public health implication considering the close associations that humans, particularly the children have built with dogs. Future research should aim at establishing the epidemiological role of dogs and possibly other animals in zoonotic enteric *E. coli* infections in humans (Khan *et al.,* 2009). Human-dog contacts should involve care so as to avoid possible transmissions of potential pathogens from these companions to humans.

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