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Occurrence and Phenotypic Antimicrobial Resistance of *Escherichia Coli and Salmonella Enterica* as Well as Coliform Load Recovered from Healthy Dogs in Tamale Metropolis, Ghana

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ABSTRACT

Salmonella enterica and *Escherichia coli* are vital bacteria associated with infections in both humans and animals. Their presence in dogs expose humans to the risk of infections. This study determined the occurrence and phenotype antimicrobial resistance of *Salmonella enterica* and *Escherichia coli* recovered from healthy dogs in the Tamale Metropolis, Ghana. The study also examined coliform loads in these dogs. A total of 120 samples from five different parts (anus, mouth, nose, inner ear and outer ear) of apparently 24 healthy dogs were examined. Isolation and antimicrobial resistance of *Salmonella enterica* and *Escherichia coli* were determined according to the Bacteriological Analytical Manual and the Disc Diffusion methods, respectively. The occurrence of *Salmonella enterica* was highest at 41.7% and lowest at 8.33% while *Escherichia coli* in the dogs was highest at 62.5% and lowest at 16.7%. The coliform load was highest at 3.7 log cfu/cm^2 and lowest at 3.1 log cfu/cm^2. The *Salmonella enterica* isolates were highly resistant to teicoplanin (100%) and tetracycline (89.5%), but susceptible to gentamicin (68.4%). The MAR index ranged from 0.1 to 0.7 and the resistance pattern TecTeCCro (resistant to teicoplanin, tetracycline, chloramphenicol and ceftriaxone) was the most common. For *Escherichia coli*, they were highly resistant to teicoplanin (84.6%) and tetracycline (73.1%), but susceptible to gentamicin (80.8%), ceftriaxone (88.5%) and chloramphenicol (92.3%). The MAR index ranged from 0.0 to 0.6 and the resistannce pattern Tec (resistant to only tetracycline) was the most common. In conclusion, this showed that apparently healthy dogs were sources of *Salmonella enterica* and *Escherichia coli*. The *Salmonella enterica* and *Escherichia coli* isolates showed varied resistances to antibiotics.

Keywords: Antimicrobial resistance, Dogs, *Escherichia coli*, Ghana, *Salmonella enterica*, Tamale

INTRODUCTION

Escherichia and *Salmonella* species are a group of bacteria of the Enterobacteriaceae family that normally inhabit the intestines of animals and humans (Janda and Abbott, 2021). Thus, most animals including dogs, cats, rabbits, rodents, reptiles, birds and livestock could be carriers or be infected by *Escherichia* and *Salmonella* species of any kind (Adzitey *et al*., 2012; Bataller *et al*., 2020; Mustapha and Goel, 2020; Valat *et al*., 2020; Wei *et al*., 2022). Mustapha and Goel (2020) had observed *Escherichia coli* in the urine of dogs, while Bataller *et al*. (2020) found that apparently healthy dogs could be sources of *Salmonella* species. *Escherichia coli* and *Salmonella* infections have been recorded around the world and are essential public health concerns (Popa and Papa, 2021; World Health Organization (WHO), 2022). The centers for Disease Control and Prevention (2021) reported an outbreak due to *Escherichia coli* which was responsible for twenty-two illnesses, eleven hospitalizations and one death. *Salmonella* caused sixteen illnesses, two hospitalizations with no deaths (CDC, 2022). According to Popa and Papa (2021), *Salmonella* was the most common cause of foodborne outbreaks in Europe.

There is evidence of transmission of *Escherichia* and *Salmonella species* from dogs to humans and vice versa (Valat *et al*., 2020; Lei *et al*., 2021). According to Lei *et al*. (2021), extended-spectrum β-lactamase genes isolated from *Escherichia coli* of dog origin were similar to those found in their owners. Major *Salmonella* STs (ST372 and ST73) found in dogs were common to those considered as human-associated (Valat *et al*., 2020). The close association between man and dogs has the potential to increase the risk of human exposure to these pathogens (Benz-Schwarzburg *et al*., 2020).

Antimicrobial resistance occurs when antibiotics are no effective in eradicating the bacteria to which they previously susceptible (Adzitey, 2015). Antimicrobial resistance is a growing public health challenge worldwide, due to the therapeutic challenges in managing humans and animals microorganisms. *Escherichia* and *Salmonella species* isolated from dogs were demonstrated to be resistant to antimicrobials such as tetracycline, chloramphenicol among others (Wei *et al*., 2020; Mustapha and Goel, 2020).

Pets including dogs are domesticated animals kept by man for companionship or pleasure (Benz-Schwarzburg *et al*., 2020). Solomon *et al*. (2019) described dogs as 'man's best friend' because they fit in with human life. Dogs are used for security, guiding the visually impaired, hunting, herding and other security purposes (Solomon *et at*., 2019; Benz-Schwarzburg *et al*., 2020). In addition, they can be trained to use their sense of smell for disease detection in human body, or illegal drugs, in the airport, crime and accident scene to help police identify or locate victims (Solomon *et al*., 2019).

In Ghana, more people keep dogs for security reasons and as pets (Adzitey *et al*., 2022). Nonetheless, data on the occurrence of Escherichia and *Salmonella species* in dogs and their antimicrobial resistance patterns in Ghana is scarce. Howbeit, the close association of dogs to their keepers exposes both to the risk of *Escherichia* and *Salmonella* infections. This study was therefore designed to determine the occurrence and phenotypic antimicrobial resistance of *Escherichia coli* and *Salmonella enterica* recovered from apparently healthy dogs in the Tamale Metropolis, Ghana. Also, the coliform loads of the various parts of the dogs were determined.

MATERIALS AND METHODS

Study area

The study was conducted in the Tamale Metropolis, Ghana. The Metropolis occupies an estimated land size of 64,690,180 sqkm and lies between latitude 9˚16 and 9˚ 34 North and longitudes 0˚ 36 and 0˚ 57

West (Ghana Statistical Service, (GSS) 2013). It has a population of 233,252 (GSS, 2013).

Sample collection

A total of 120 samples were collected from 24 apparently healthy dogs from different locations in the Tamale metropolis. Sterile cotton swabs were used to take five samples each from the anus, mouth, nose, inner ear and outer ear of each dog. The swabbed samples were placed in Coleman box containing ice blocks and subsequently transported to the Spanish Laboratory, at the University for Development Studies, Nyankpala, for microbial analysis.

Enumeration of coliform bacteria count

This was done using a slightly modified procedure of Maturin and Peeler (2001) and Adzitey *et al*. (2020). The samples were dipped in 10 ml of 1% buffered peptone water (BPW, Oxoid Limited, Basingstoke, UK) and serial dilutions from 10^{-1} to 10^{-5} were made. Serially diluted samples were spread plated (0.1 ml) onto MacConkey agar and incubated at overnight at 37°C. Colonies were then counted and expressed in colony forming units.

Isolation and identification of *E. coli*

Swabs were dipped in 9ml of BPW and incubated overnight at 37℃. Overnight aliquots were streaked onto Levine Eosin Methylene Blue agar (LEMB Oxoid Limited, Basingstoke, UK) and incubated at 37℃ for 18-24 hours (Feng *et al*., 2020). Presumptive *E. coli* isolates forms colonies with a green metallic sheen and a dark nucleated core on LEMB. Such isolates were purified and confirmed using Gram stain and *E. coli* latex agglutination test by following the manufacturer's instructions.

Isolation and identification of *Salmonella enterica*

A slightly modified procedure of Andrews *et al*. (2014) was used. Overnight aliquots in BPW incubated at 37°C were enriched in Rappaport-Vassiliadis broth, Oxoid Limited, Basingstoke, UK (incubated at 42°C for 24h) and Selenite Cystine broth, Oxoid Limited, Basingstoke, UK (incubated at 37°C for 24h). After which, the aliquots were streaked on Xylose Lysine Deoxycholate and Brilliant Green agar. Presumptive *Salmonella enterica* were purified and confirmed using gram stain, triple sugar iron agar, lysine iron agar, and *Salmonella* latex agglutination test by following the manufacturer's instructions.

PCR for the confirmation of *E. coli* and *Salmonella enterica* isolates

DNA template was prepared by lysing colonies of *E. coli* and *Salmonella enterica* in 30µl DNase/RNase free water at 99℃ for 30min (Kichana *et al*., 2022). Conventional PCR was carried out to amplify partial Invasion A (*invA*) gene of *S. enterica* by following the procedures of Bej *et al*. (1999) and partial Beta-D-glucuronidase (*uidA*) gene of *E. coli* by following the procedures of Malorny *et al*. (2002) in a 50µl reactions. Briefly, the reaction consisted of 10µM each of respective primer pairs (F: AAA ACG GCA AGA AAA AGC AG, R: ACG CGT GGT TAC AGT CTT GCG for *E. coli* and F: GTG AAA TTA TCG CCA CGT TCG GGC AA, R: TCA TCG CAC CGT CAA AGG AAC C for *Salmonella enterica*), 1.8mM $MgCl_2$, 20mM Tris-HCl (pH 8.9 at 25°C), 22mM NH_4Cl, 0.2mM dNTPs, 5% glycerol, 0.06% IGEPAL® CA-630, 0.05% Tween-20, xylene Cyanol FF, Tartrazine, 1.25U One Taq® DNA polymerase (New England Biolabs® Inc) and 4µl DNA template. The cycling conditions were initial denaturation at 95℃ for 5 min, followed by 35 cycles of denaturation at 95℃ for 30 s, annealing at 57℃ for 30s and extension at 72℃ for 5min 30 s. The PCR amplicons were resolved on a 2% (w/v) agarose gel stained with EtBr and fragment size estimated by comparing with FastRuler™ Middle Range DNA Ladder [10mM Tris-HCl (pH 7.6), 10mM EDTA, 0.005% bromophenol blue and 10% glycerol].

Antimicrobial resistance of *E. coli* and *Salmonella enterica*

Antimicrobial resistance test was done according to Bauer *et al*. (1966). The isolates were examined against; amoxicillin/clavulanic acid (Amc) 30 µg, azithromycin (Azm) 15 µg, ceftriaxone (Cro) 30 µg, chloramphenicol (C) 30 µg, ciprofloxacin (Cip) 5 µg, gentamicin (Cn) 10 µg, sulfamethoxazole/trimethoprim (Sxt) 22 µg, teicoplainin (Tec) 30 µg and tetracycline (Te) 30 µg purchased from Oxoid Limited, Basingstoke, UK. The cultures were incubated in tryptic soy broth at 37ºC for 18 h and the concentration adjusted to 0.5 McFarland turbidity. It was then spread plated on Mueller Hinton agar, Oxoid Limited, Basingstoke, UK (MH) and incubated at 37°C for 24 h. The inhibition zones were measured and the results were interpreted according to Clinical Laboratory Standard Institute (2022). The Multiple Antibiotic resistance (MAR) index was calculated and interpreted according to Krumperman (1983) using the formula: a/b, where 'a' represents the number of antibiotics to which a particular isolate was resistant and 'b' the total number of antibiotics tested.

Statistical analysis

Coliform count was analyzed using ANOVA of GenStat 12.1 Edition. The means were separated at 5% significant level. Occurrence data was analyzed using binary logistic of IBM Statistical Package for the Social Sciences (SPSS) Version 17. Test for statistical difference was done using wald chi-square at 5% significance level.

RESULTS

Coliform counts of healthy dogs in the Tamale metropolis

Table I shows the coliform counts of the anus, mouth, nose, inner ear and outer ear of the apparently healthy dogs in the Tamale metropolis. The coliform count was 3.7 log cfu/cm^2, 3.3 log cfu/cm^2, 3.3 log cfu/cm^2, 3.2 log cfu/cm^2 and 3.1 log cfu/cm^2 for anus, mouth, nose, inner ear and outer ear, respectively. There were no significant differences p value 0.139 in coliform counts among the anus, mouth, nose, inner ear and outer ear samples examined.

TABLE I: Coliform count of samples from dogs

Source	Coliform count (log cfu/cm^2)
Anus	3.7
Mouth	3.3
Nose	3.3
Inner ear	3.2
Outer ear	3.1
Sed	0.179
P-value	0.139

Incidence of *Escherichia coli* in apparently healthy dogs

The incidence of *Escherichia coli* in the apparently healthy dogs in the Tamale Metropolis is presented in Table II. The overall incidence of *Escherichia coli* in the dogs was 45% (54/120). *Escherichia coli* was most common in the mouth (62.5%), followed by anus (58.3%), nose (45.8%), outer ear (41.7%) and inner ear (16.7%). Significant differences ($P<0.05$) occurred among the incidence of *Escherichia coli* in the dog samples analyzed. Mouth, anus, nose, and outer ear samples positive for *Escherichia coli* did not differ significantly

(*P>0.05*) from each other, but were significantly higher *(P<0.05)* than those from inner ear samples.

TABLE II: Incidence of *Escherichia coli* in apparently healthy dogs

Sources	Number of samples tested	Positive samples	Incidence %
Anus	24	14	58.3
Mouth	24	15	62.5
Nose	24	11	45.8
Inner ear	24	4	16.7
Outer ear	24	10	41.7
Overall	120	54	45.0

Incidence of *Salmonella enterica* in apparently healthy dogs

The incidence of *Salmonella enterica* in apparently healthy dogs in the Tamale metropolis is shown in Table III. The overall incidence of *Salmonella enterica* in healthy dogs was 24.2% (29/120). *Salmonella enterica* was isolated from the anus (41.7%), mouth (33.3%), nose (25.0%), outer ear (12.5%) and inner ear (8.3%). Significant differences (*P<0.05*) occurred among the incidence of *Salmonella enterica* in the dog samples analyzed. Anus samples positive for *Salmonella enterica* did not differ (*P>0.05*) from that of mouth and nose, but were significantly higher (*P<0.05*) than those obtained from the outer and inner ears. Mouth samples positive for *Salmonella enterica* were significantly higher (*P<0.05*) than those obtained from the inner ear, but not nose and outer ear samples. Nose, inner and outer ears positive for positive for *Salmonella enterica* did not differ (*P>0.05*) from each other.

TABLE III: Incidence of *Salmonella enterica* in apparently healthy dogs

Source	Number of samples tested	No. positive	Incidence %
Anus	24	10	41.7
Mouth	24	8	33.3
Nose	24	6	25.0
Inner Ear	24	2	8.3
Outer Ear	24	3	12.5
Total	120	29	24.2

Antimicrobial susceptibility of *Escherichia coli* isolated from dogs in Tamale

The antimicrobial resistance of *Escherichia coli* isolated from dogs are presented in Table IV. The overall resistant, intermediate resistant and susceptible was 29.9%, 11.5% and 58.6%, respectively. The highest resistance occurred for teicoplanin (84.6%), followed by tetracycline (73.1%). The isolates showed high susceptibility to chloramphenicol (92.3%), ceftriaxone (88.5%) and gentamicin (80.8%). Relatively higher intermediate resistances were observed for ciprofloxacin (30.8%), azithromycin (23.1%), gentamicin (19.2%) and tetracycline (19.2%). Intermediate resistance *Escherichia coli* isolates are those that are neither completely resistant nor susceptible and can be difficult to treat when they are involved in infections (Lorian, 2005; Adzitey *et al*., 2020).

TABLE IV: Antimicrobial susceptibility of *Escherichia coli* isolated from dogs in Tamale

Antimicrobials	% Resistant	% Intermediate resistant	% Susceptibility
Amoxicillin/clavulanic acid 30 µg	42.3	0.0	57.7
Azithromycin 15 µg	15.4	23.1	61.5
Ceftriaxone 30 µg	0.0	11.5	88.5
Chloramphenicol 30 µg	7.7	0.0	92.3
Ciprofloxacin 5 µg	3.8	30.8	65.4
Gentamicin 10 µg	0.0	19.2	80.8
Teicoplanin 30 µg	84.6	0.0	15.4
Tetracycline 30 µg	73.1	19.2	7.7
Sulphamethoxazole/trimethoprim 22 µg	42.3	0.0	57.7
Overall	29.9	11.5	58.6

Key: Amoxicillin/clavulanic acid (Amc) 30 µg, Chloramphenicol (C) 30 µg, Gentamicin (Cn) 10 µg, Ceftriaxone (Cro) 30 µg, Ciprofloxacin (Cip) 5 µg, Azithromycin (Azm) 15 µg, sulfamethoxazole/trimethoprim (Sxt) 22 µg, Tetracycline (Te) 30 µg and Teicoplainin (Tec) 30 µg.

Antimicrobial susceptibility of *Salmonella enterica* isolated from dogs in Tamale

From Table V, *Salmonella enterica* isolates from the dog samples exhibited an overall resistance, intermediate resistance and susceptibility of 49.1%, 11.8% and 39.1%, respectively. The *Salmonella enterica* isolates were highly resistance to teicoplanin (100%), followed by tetracycline (89.5%). Gentamicin, ciprofloxacin and sulfamethoxazole/trimethoprim had the highest susceptibility of 68.4%, 63.2% and 63.2%, respectively. Intermediate resistance was prominent for gentamicin (26.3%), ciprofloxacin (26.3%) and ceftriaxone (21.8%). Intermediate resistant *Salmonella enterica* isolates can alter treatment patterns when they are involved in infections. Lorian, 2005; Adzitey *et al*., 2020).

Antimicrobial resistance profile and multiple antibiotic resistance (MAR) index of individual *Escherichia coli* from apparently healthy dogs' origin

The antimicrobial resistance profile and MAR index of *Escherichia coli* isolated from the apparently healthy dogs can be seen in Table VI. Two, five and eleven *Escherichia coli* isolates were resistant to five, four and three different antimicrobials, respectively. Therefore, multidrug resistance (resistant to 3 or more different antimicrobials) was recorded for 18 isolates (69.2%). In this study, 5 (19.2%), 4 (15.4%), 3 (11.5%), 2 (7.7%), 1 (3.8%) and 0 (0.0%) showed resistance to two, five, eleven, one, one and one antimicrobials. Multiple antibiotic resistance (MAR) ranged from 0 to 0.56, that is, resistant to 0 and 5 antimicrobials, respectively.

TABLE V: Antimicrobial susceptibility of *Salmonella enterica* isolated from dogs in Tamale

Antimicrobial	R%	I%	S%
Amoxicillin/clavulanic acid 30µg (Amc)	42.1	0.0	57.9
Azithromycin 15µg (Azm)	63.2	15.8	21.0
Ceftriaxone 30µg (Cro)	47.4	21.8	30.8
Chloramphenicol 30µg(C)	52.6	0.0	47.4
Ciprofloxacin 5µg (Cip)	10.5	26.3	63.2
Gentamicin10µg (Cn)	5.3	26.3	68.4
Teicoplanin 30 µg (Tec)	100	0.0	0.0
Tetracycline 30µg (Te)	89.5	10.5	0.0
Sulfamethoxazole/trimethoprim (Sxt)	31.6	5.2	63.2
Overall%	49.1	11.8	39.1

Key: Amoxicillin/clavulanic acid (Amc) 30 µg, Chloramphenicol (C) 30 µg, Gentamicin (Cn) 10 µg, Ceftriaxone (Cro) 30 µg, Ciprofloxacin (Cip) 5 µg, Azithromycin (Azm) 15 µg, Sulfamethoxazole/trimethoprim (Sxt) 22 µg, Tetracycline (Te) 30 µg and Teicoplainin (Tec) 30 µg.

TABLE VI: Antimicrobial resistannce profile and multiple antibiotic resistant (MAR) index of individual *Escherichia coli*

Sources	No. of Antibiotics	Antibiotic resistance profile	MAR index
Outer ear	5	CipAmcTecTeSxt	0.56
Outer ear	5	AmcTecTeCSxt	0.56
Anus	4	AmcTecTeSxt	0.44
Mouth	4	AzmCnTeSxt	0.44
Nose	4	AmcTecTeSxt	0.44
Outer ear	4	TecTeCSxt	0.44
Outer ear	4	AmcTecTeSxt	0.44
Anus	3	AmcTecTe	0.33
Anus	3	AmcTecTe	0.33
Mouth	3	AmcTecTe	0.33
Mouth	3	AmcAzmTec	0.33
Mouth	3	TecTeSxt	0.33
Nose	3	AmcTecTe	0.33
Nose	3	AzmTecTe	0.33
Nose	3	TecTeSxt	0.33

Outer ear	3	TecTeSxt	0.33
Outer ear	3	TecTeSxt	0.33
Inner ear	3	AzmTecTe	0.33
Inner ear	2	TecTe	0.2
Anus	1	Tec	0.11
Anus	1	Te	0.11
Anus	1	Tec	0.11
Mouth	1	Tec	0.11
Mouth	1	Amc	0.11
Nose	1	Tec	0.11
Inner ear	0	All susceptible	0.00

Key: Amoxicillin/clavulanic acid (Amc) 30 µg, Chloramphenicol (C) 30 µg, Gentamicin (Cn) 10 µg, Ceftriaxone (Cro) 30 µg, Ciprofloxacin (Cip) 5 µg, Azithromycin (Azm) 15 µg, Sulfamethoxazole/trimethoprim (Sxt) 22 µg, Tetracycline (Te) 30 µg and Teicoplainin (Tec) 30 µg.

TABLE VII: Antimicrobial resistance profile and multiple antibiotic resistance (MAR) index of individual *Salmonella enterica*

Source	No. of Antibiotics	Antibiotic resistant profile	MAR index
Nose	6	CipAzmTecTeCSxt	0.67
Mouth	6	AmcAzmTecTeCCro	0.67
Mouth	6	AzmTecTeCCroSxt	0.67
Anus	6	AmcTecTeCCroSxt	0.67
Anus	6	CipAzmTecTeCSxt	0.67
Mouth	5	AzmTecCnTeCro	0.56
Inner ear	5	AmcAzmTecTeCro	0.56
Anus	5	AmcAzmTecTeC	0.56
Anus	5	AzmTecTeCSxt	0.56
Anus	5	AmcAzmTecTeC	0.56
Anus	5	AmcAzmTecTeSxt	0.56
Nose	4	TecTeCCro	0.44
Inner ear	4	TecTeCCro	0.44
Anus	4	TecTeCCro	0.44
Anus	4	AmcTecTeCro	0.44
Mouth	3	AzmTecTe	0.33
Mouth	3	AzmTecTe	0.33
Inner ear	3	AmcTecTe	0.33
Outer ear	1	Tec	0.11

Key: Amoxicillin/clavulanic acid (Amc) 30 µg, Chloramphenicol (C) 30 µg, Gentamicin (Cn) 10 µg, Ceftriaxone (Cro) 30 µg, Ciprofloxacin (Cip) 5 µg, Azithromycin (Azm) 15 µg, sulfamethoxazole/trimethoprim (Sxt) 22 µg, Tetracycline (Te) 30 µg and Teicoplainin (Tec) 30 µg.

Antimicrobial resistance profile and multiple antibiotic resistance (MAR) index of individual *Salmonella enterica* from apparently healthy dogs' origin

The antimicrobial resistance profile and MAR index of *Salmonella enterica* isolated from the apparently healthy dogs can be seen in Table VII. Five, six, four and three *Salmonella enterica* isolates were resistant to six, five, four and three different antimicrobials, respectively. Thus, multidrug resistance (resistant to 3 or more different antimicrobials) was recorded for 18 isolates (94.7%). Therefore, 94.7% of the *Salmonella enterica* isolates originated from sources where they were frequently exposed to antimicrobials as indicated by Rotchell *et al*. (2016). Multiple antibiotic resistance (MAR) ranged from 0.11 to 0.67, indicating, resistance to 1 and 6 antimicrobials, respectively.

DISCUSSION

In Ghanaian culture, the number of owned dogs has increased dramatically bringing dogs in close contact with humans. Such close contacts facilitate the transmission of pathogens between pets and humans. This study showed that coliforms were present in the dog samples analyzed. Numerically, coliform counts were highest in the anus and least in the outer ear. These coliforms could be naturally present in the parts of the apparently healthy dogs examined or the dogs might have picked them from their feeds, environment and humans (Benz-Schwarzburg et al., 2020; Adzitey et al., 2022). For instance, the occurrence of coliforms in the outer ear is a sign of cross contamination from other parts of the dogs, their feeds, environment or humans since outer ear is not natural habitat for coliforms. Coliforms are a large group of bacteria of different types and can be harmful, although most are harmless. They can be found in the environment, soils, humans and animal faeces (Maturin and Peeler, 2001; Feng et al., 2020). According to Martin et al. (2016), the presence of coliforms suggests that there could be pathogenic microbes and includes Escherichia coli, Citrobacter, Klebsiella, Hafnia and Enterobacter. Cinquepalmi et al. (2013) found various types of bacteria in dogs faces collected from urban streets and indicated they serve as a risk factor for the transmission of microorganisms among humans, the environment and dogs. Ruparell et al. (2020) observed diverse bacterial species in the mouth of dogs. Dog feeds have also been reported as potential sources of bacteria that can cross contaminate the mouth and other parts of the dog (Morelli et al., 2020). Strains of Escherichia coli harbor the gastrointestinal tract of dogs and can cause watery diarrhea, vomiting, loss of appetite, fever and kidney failure (Feng et al., 2020). The presence of Escherichia coli in the inner and outer ears could be due to cross contamination as a result of dogs lying on the floor, licking their bodies, contact with humans among others. In Australia, Roberts et al. (2019) found that Escherichia coli (64.0%) was the most prevalent pathogen in the urinal tract of canine, which was higher than the overall incidence of 45.0% found in this study. In Ethiopia, Gebremedhin et al. (2021), reported that rectal swabs from dogs in Bako (31.0%), Gojo (31.0%) and Ambo (18.5%) towns were contaminated with Escherichia coli, comparatively a higher incidence rate of 58.3% was found for the anus. The overall prevalence of Escherichia coli in the rectal swabs of the dogs was 24.2% (Gebremedhin et al., 2021), which was lower than that of the present study which was 45%. In Nigeria, Azuonwu et al. (2022) found that 20.0% of dog stools 313 were contaminated with Escherichia coli which was also lower than the 58.3% observed in this 314 study. In UK, Groat et al. (2022) analysed 190 dog faecal samples and found 31 samples (16.3%) 315 to be positive for Escherichia coli. This study found a higher prevalence Escherichia coli in dog 316 faeces. 317 318 Dogs are companion animals and exhibit closeness to humans. They can serve as sources of 319 resistant Escherichia coli which can be transferred to humans. Therefore, care must be taking in 320 handling dogs to prevent such infections. Comparable to this work, other workers have reported 321 on the presence of Escherichia coli in dogs. Robert et al. (2019) reported that Escherichia coli 322 isolated from dogs were resistant to amoxicillin/clavulanic acid (22%) and 323 sulphamethoxazole/trimethoprim (8%), which were lower than what was observed in this study. 324 Gebremedhin et al. (2021) found that, Escherichia coli from dog faeces were 14.0% resistant to 325 ceftriaxone, 11.9% resistant to gentamicin and 7% resistant to sulphamethoxazole/trimethoprim. 326 This study found no resistance to ceftriaxone and gentamicin, but higher resistance to 327 11 sulphamethoxazole/trimethoprim. Escherichia coli of dogs' origin were resistant to azithromycin 328 (25.7%), amoxicillin/clavulanic acid (5.7%), ceftriaxone (20.0%), gentamicin (8.6%) and 329 tetracycline (48.6%) (Ventura et al., 2024). Higher resistances to tetracycline and 330 amoxicillin/clavulanic acid were reported in this study, but no or lower resistances were reported 331 for ceftriaxone, gentamicin and azithromycin. Leonard et al. (2012) indicated that Escherichia coli 332 from dogs were 6.8%, 4.5% 3.0% 9.0%, 0.8% 3.0% and 15.8% resistant to amoxicillin/clavulanic 333 acid, ceftriaxone, ciprofloxacin, trimethoprim/sulfamethoxazole, gentamicin, chloramphenicol 334 and tetracycline, respectively, which are comparable to the current study. 335 336 Multidrug resistance Escherichia coli isolates have also been reported in dogs (Leonard et al., 337 2012; Robert et al., 2019; Gebremedhin et al., 2021; Ventura et al., 2024). Ventura et al. (2024) 338 reported that four (4) Escherichia coli isolates from dogs did not present resistance to any 339 antimicrobial; this study also found that one Escherichia coli isolated from inner ear presented no 340 resistance to any of the antimicrobials examined. Furthermore, 3 (8.57%), 5 (14.28%), 3 (8.57%), 341 1 (2.86%) and 5 (14.28%) Escherichia coli isolates showed resistance to seven, six, four, three, 342 two and one antimicrobial agents of different groups (Ventura et al., 2024). Resistance to three or 343 more antimicrobials were detected in 24 (96%) Escherichia coli isolates of dog origin (Wei et al., 344 2020), which was 69.2% in the present study. Bacteria with MAR index of greater than 0.2 345 originate from sources where antibiotics are frequently used, while those with MAR less than 0.2 346 originate from sources where antimicrobial usage is uncommon (Rotchell et al., 2016). Based on 347 this, 69.2% of the isolates originated from sources where antimicrobials are frequently used. The 348 Escherichia coli isolates exhibited 13 different phenotypic antimicrobial resistant profiles. The 349 resistant profile AmcTecTe, Tec and TecTeSxt were the most common and was exhibited by 4 350 different Escherichia coli isolates each. Escherichia coli isolated from the outer ears showed the 351 highest number of resistances, that is, resistant to CipAmcTecTeSxt and AmcTecTeCSxt. Also, 352 Escherichia coli from the anus, nose and outer ear shared the same resistance pattern of 353 AmcTecTeSxt. This suggests possible cross contamination, but a more robust method comparing 354 the DNA sequences of the Escherichia coli will provide better proof. 355 356 357 Salmonella enterica like Escherichia coli live in the intestinal tract of dogs and can be shed during 358 defecations. They cause infections with symptoms such as nausea, fever, headache, chills and 359 bloody stools (Andrews et al., 2014). Salmonella enterica was highest in samples collected from 360 dog anus, followed by mouth, nose, outer ear and inner ear. The presence of Salmonella enterica 361 in the mouth, nose, inner and outer ear samples of dog signify cross contamination from the anus 362 or environment since these sources may not be natural habitats of Salmonella enterica. The results 363 of this study are comparable to others. Groat et al. (2022) in the UK detected that, 8 (4.2%) out of 364 190 dog faecal samples analysed were positive for Salmonella species. In China, Wei et al. (2020) 365 reported that, 9.5% of dog faeces were positive for Escherichia coli. Kiflu et al. (2017) examined 366 360 apparently healthy dogs in Ethiopia and reported that 11.7% were positive for Salmonella 367 species. Reimschuessel et al. (2017) in the USA reported a prevalence of 2.5% for Salmonella 368 species isolated from dog faecal samples. They also found that diarrhea was present in 55.0% of 369 the dogs and rural dogs were more likely to be positive for Salmonella compared to urban and 370 suburban dogs. In New Zealand, Bojanić et al. (2022) examined dog faecal samples and found no 371 Salmonella species. The prevalence of Salmonella enterica in the anus of dogs in this study were 372 12 higher than that reported by Kiflu et al. (2017), Reimschuessel et al. (2017); Wei et al. (2020) and 373 Groat et al. (2022). 374 375 Dogs especially those that are used as pets associate closely with their owners and the use of 376 antibiotics for prophylactics and treatment of sick dogs expose them to developing antimicrobial 377 resistances to pathogens including Salmonella enterica. Resistant Salmonella enterica isolates can 378 be transferred to humans via improper handling. Evidence of antimicrobial resistant Salmonella 379 species in some dogs are available. Leonard et al. (2012) found that Salmonella species from dogs 380 were 12.5%, 12.5%, 0.0%, 0.0%, 0.0% 0.0% and 3.1% resistant to amoxicillin/clavulanic acid, 381 ceftriaxone, ciprofloxacin, sulphamethoxazole/trimethoprim, gentamicin, chloramphenicol and 382 tetracycline, respectively, which were all lower than that what was found in this study. Wei et al. 383 (2020) reported that, Salmonella species of dog origin were resistant to tetracycline (92%), 384 azithromycin (88%) and ceftriaxone (80%), which were higher than the resistances observed in 385 this study. Usmael et al. (2022) subjected Salmonella species isolated from dog sources to 386 antimicrobial sensitivity test and found resistant, intermediate resistant and susceptibility of 387 21.2%, 4.2% and 66.6%, 12.5%, 29.2% and 58.3%, and 4.2%, 4.2% and 58.3% to tetracycline, 388 amoxicillin/clavulanic acid and sulphamethoxazole/trimethoprim, respectively. This study found 389 resistant, intermediate resistant and susceptibility of 89.5%, 10.5% and 0.0%, 42.1%, 0.0% and 390 57.9%, and 31.6%, 5.2% and 63.2% to tetracycline, amoxicillin/clavulanic acid and 391 sulphamethoxazole/trimethoprim, respectively. Jajere et al. (2014) reported that, Salmonella 392 isolates from fecal samples of dogs were susceptible to ciprofloxacin (89.7%), 393 amoxicillin/clavulanic acid (87.6%) and chloramphenicol (84.5%) and this result found 63.2% 394 susceptibility to ciprofloxacin, 57.9%

susceptibility to amoxicillin/clavulanic acid and 47.4% 395 susceptibility to chloramphenicol. A resistance rate of 26.2% to amoxycillin/clavulanic was found 396 in Escherichia coli isolated from dog faeces (Kiflu et al., 2017), which was lower than the 42.1% 397 observed in the current study. 398 399 Salmonella enterica that are resistant to multiple antimicrobials have also been reported (Leonard 400 et al., 2012; Jajere et al. 2014; Wei et al., 2020; Usmael et al., 2022). The Salmonella enterica 401 isolates exhibited 16 different phenotypic antimicrobial resistant profiles. The resistant profile 402 TecTeCCro was the most common and was exhibited by 3 different Salmonella enterica isolates 403 each. Salmonella enterica isolated from the nose, inner ear and anus exhibited that same resistant 404 pattern of TecTeCCro, which could be due to cross contamination. Kiflu et al. (2017) reported that 405 38 (90.5%) Salmonella isolates were resistant to one or more antimicrobials, 30 (71.4%) were 406 resistant to two or more antimicrobials, 19 (45.2%) were resistant to three or more antimicrobials, 407 10 (23.8%) were resistant to five or more antimicrobials and 4 (9.5%) were resistant to eight or 408 more antimicrobials tested. In this study, 18 (94.7%) of the Salmonella enterica isolates were 409 resistant to three or more antimicrobials. Resistance to zero, one, two and three different 410 antimicrobials were 41.7%, 37.5%, 12.5% and 8.3%, respectively (Usmael et al., 2022), while 411 resistance to zero, one, two and three different antimicrobials were 0.0%, 5.3%, 0.0%, and 15.8%, 412 respectively in the current study.

CONCLUSION

The anus, mouth, nose and ear of dogs are potential sources of coliforms, *Escherichia coli* and *Salmonella enterica*. The *Escherichia coli* and *Salmonella enterica* isolates exhibited varying level of resistance and susceptibility to antimicrobials. To the best of our knowledge this study reports for the first time on the occurrence and phenotypic antimicrobial resistance of *Escherichia coli* and *Salmonella enterica* recovered from healthy dogs in the Tamale Metropolis, Ghana. The incidence of resistant *Escherichia coli* and *Salmonella enterica* in dogs in the study area means that humans are exposed to the risk of contracting infections that will be difficult to treat from these pathogens which is a threat to public health. Therefore, dog owners should keep their dogs under hygienic environmental conditions, including providing dogs with safe foods, preventing dogs from scavenging or roaming for food and regularly checking their pets' health status/vaccinations. Also, thorough hand washing with soap and water after touching pets is recommended.

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