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Antibiotic Susceptibility of *Escherichia coli* Isolated from some Drinking Water Sources in Tamale Metropolis of Ghana

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ABSTRACT

This is the first report on the antibiotic resistance of *Escherichia coli* isolated from drinking water samples in Tamale Metropolis of Ghana. Antibiotic susceptibility test was performed using the disc diffusion method and the results interpreted using the Clinical and Laboratory Standards Institute guidelines. A total of 56 water sample *Escherichia coli* isolates were screened against nine different antibiotics. Overall, 37.90% of the *Escherichia coli* isolates were resistant, 12.90% were intermediate and 49.21% were susceptible. Resistance to vancomycin (94.64%) and erythromycin (85.71%) was high. Susceptibility to ciprofloxacin (94.64%), gentamicin (91.07%) and ceftriaxone (89.29%) was also high. A relatively higher percentage of the water sample *Escherichia coli* isolates exhibited intermediate resistance to amoxycillin/clavulanic acid (50%). The *Escherichia coli* isolates also exhibited 24 antibiotic resistant patterns with the pattern E-VA (erythromycin-vancomycin) and SXT-E-VA-TE (Trimethoprim/sulfamethoxazole-erythromycin-vancomycin-tetracycline) being the commonest (each exhibited by nine different isolates). Multiple antibiotic index (MAR index) ranged from 0.11-0.56. Resistance to five (MAR index of 0.56) and four (MAR index of 0.44) different antibiotics were exhibited by 8 and 21 isolates, respectively. A number of *Escherichia coli* isolated from different water sources did exhibit the same resistant pattern. This study revealed that *Escherichia coli* from drinking water sources in Tamale, Metropolis are resistant to some antibiotics. Therefore, the use of antibiotics in animal production and for human treatments in the Metropolis need to be checked and curbed to prevent more isolates from becoming resistant.

Key words: Antibiotics, *Escherichia coli*, drinking water, susceptibility, resistance

INTRODUCTION

Water is very essential for life. Both humans and animals need water to survive since all cellular activities take place in fluid medium. Water constituents about seventy five percent 75% in newborns and 50% in mature animals of which 2/3 are found inside the cell. Blood, which contains 80% water, is vital in transporting oxygen to the tissues and carbon dioxide from the tissues as well as being the life support system for the body. Visually clear and colorless drinking water is acceptable, however, it should be safe and free from chemical toxin and pathogenic microorganisms (Maheshwari, 2008; Adzitey *et al.*, 2015a). Humans and animals drink water from a variety of sources including rain water, wells, tap water, sachet water, bottle water, well water, dam water, water from the drinking troughs etc some of which can be contaminated by *Escherichia coli*, other pathogens and chemical toxins (Maheshwari, 2008; Carnot *et al.*, 2014; Anita *et al.*, 2014; Adzitey *et al.*, 2015a).

Besides water samples, *Escherichia coli* have been isolated from farm animals, wild animals, pests, humans, vegetables and many more (Shakak and Saeed, 2010; Abulreesh, 2011; Adzitey *et al.*, 2011, 2012a, 2013, 2014, 2015b; Manikandan *et al.*, 2011; Geidam *et al.*, 2012; Kumar *et al.*, 2013; Al-Sultan *et al.*, 2014; Biswas *et al.*, 2014). *Escherichia coli* have been responsible for a number of foodborne outbreaks, diseases and deaths in some cases (CDC., 2014). Antibiotic resistance is a global problem and new forms of antibiotic resistance can cross international boundaries with ease. The use of antibiotics in agriculture and human treatments will continue to increase especially in regions where people have less knowledge in its negative effects. The use of antibiotic is a main driver of selection pressure that contributes to resistance (Aarestrup *et al.*, 2008; Adzitey *et al.*, 2012b). The causes of antibiotic resistance are complex and include human behaviour at many levels of society; the consequences affect everybody in the world (Laxminarayan *et al.*, 2013).

Work on the antibiotic resistance of *Escherichia coli* isolated from water sources in Tamale is unavailable. Therefore, this work was carried out to determine the antibiotic resistance of *Escherichia coli* isolated from some drinking water sources for humans and animals in Tamale Metropolis of Ghana.

MATERIALS AND METHODS

Sources of *Escherichia coli* isolates: Fifty six *Escherichia coli* isolated from drinking water sources for humans and animals between August 2013 to January 2014 in the Tamale Metropolis of Ghana was used for this study. The *Escherichia coli* isolates were obtained from tap water (n = 9), rain water (n = 10), well water (n = 10), dam water (n = 11) and water from the drinking troughs of poultry (n = 10) and ruminant (n = 6).

Antimicrobial susceptibility of *Escherichia coli*: The disk diffusion method of Bauer *et al.* (1966) was used to determine the antibiotic resistance of 56 *Escherichia coli* against the following antibiotics; amoxicillin/clavulanic acid (AMC) 30 µg; chloramphenicol (C) 30 µg; gentamicin (CN) 10 µg; ceftriaxone (CRO) 30 µg; ciprofloxacin (CIP) 5 µg; erythromycin (E) 15 µg; sulphamethoxazole/Trimethoprim (SXT) 22 µg; tetracycline (TE) 30 µg and vancomycin (VA) 30 µg. The disks were purchased from Oxoid Limited, Basingstoke, UK. Pure cultures of *Escherichia coli* were grown overnight in Tryptic Soy Broth (TSB) (Oxoid Limited, Basingstoke, UK) at 37°C and the concentration adjusted using sterile TSB until a 0.5 McFarland turbidity was attained. One hundred microliter of the culture was then swabbed onto Mueller Hinton agar (Oxoid Limited, Basingstoke, UK) using a sterile cotton swab. Three antimicrobial disks were placed on the surface of the agar plate at a distance to avoid overlapping of inhibition zones. The plates were incubated at 37°C for 16-18 h and the results were interpreted as sensitive, intermediate, or resistance according to Clinical and Laboratory Standards Institute guidelines for (CLSI., 2006). The Multiple Antibiotic Resistance (MAR) index was calculated and interpreted according to Krumpferman (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.

RESULTS AND DISCUSSION

Fifty six *Escherichia coli* isolated from drinking water for humans and animals in the Tamale Metropolis of Ghana were tested for their antimicrobial susceptibility against 9 antibiotics. The result obtained for the antimicrobial susceptibility test is shown in Table 1. From Table 1, the

Table 1: Percentage antibiotic resistance of *Escherichia coli* isolated from drinking water samples in Tamale, Metropolis

Antibiotics	Dose (µg)	Susceptible (%)	Intermediate (%)	Resistance (%)
Amoxicillin/clavulanic acid (AMC)	30	42.46	50.00	7.14
Ceftriaxone (CRO)	30	89.29	5.36	5.36
Chloramphenicol (C)	30	51.79	17.86	30.36
Ciprofloxacin (CIP)	5	94.64	3.57	1.79
Erythromycin (E)	15	0.00	14.29	85.71
Gentamicin (CN)	10	91.07	3.57	5.36
Suphamethoxazole/Trimethoprim (SXT)	22	41.07	5.36	53.57
Tetracycline (TE)	30	30.36	12.50	57.14
Vancomycin (VA)	30	1.79	3.57	94.64

overall susceptibility, intermediate and resistance was 49.21% (248/504), 12.90% (65/504) and 37.90% (191/504), respectively. A large percentage of the *Escherichia coli* were susceptible to ciprofloxacin (94.64%), gentamicin (91.07%) and ceftriaxone (89.29%) but resistant to vancomycin (94.64%) and erythromycin (85.71%). Intermediate resistances were observed for all the antibiotics and it was relatively higher for amoxicillin/clavulanic acid (50%). Intermediate resistance refers to those *Escherichia coli* isolates that were not clearly resistant or susceptible, and such isolates have the tendency to easily become resistant (Adzitey *et al.*, 2012b; Adzitey, 2015). It is also indicated that in clinical diagnoses patients with intermediate results can be given a higher dosage of antibiotics. Antibiotics play very important role in decreasing diseases, illness and/or death associated with bacterial infections in humans and animals. Nonetheless the use of antibiotics as growth promoters and therapeutics purposes have been the major driving force behind the emergence and spread of drug-resistance bacteria among pathogenic and non-pathogenic bacteria strains (Krumperman, 1983; Aarestrup *et al.*, 2008; Adzitey *et al.*, 2012b). The main reservoir of *Escherichia coli* is the gastrointestinal tract of animals (Aarestrup *et al.*, 2008; Frederick, 2011). Through poor processing and handling of farm animals *Escherichia coli* can contaminate a variety of sources including drinking water (Adzitey *et al.*, 2015a). Humans and farm animals can get *Escherichia coli* infection by drinking water from such sources (Adzitey *et al.*, 2015a).

Antibiotic resistance profile and Multiple Antibiotic Resistance (MAR) index of the individual *Escherichia coli* are presented in Table 2. From Table 2, the drinking water *Escherichia coli* isolates exhibited 24 antibiotic resistant patterns with MAR index ranging from 0.11-0.56. One *Escherichia coli* (R10) isolated from rain water stored in a tank was susceptible to all the antibiotics. The majority of the *Escherichia coli* (21 isolates) were resistant to four antibiotics (MAR index of 0.44), followed by resistant to three antibiotics (17 isolates; MAR index of 0.33), resistant to two (9 isolates; MAR index of 0.22) and resistant to 5 antibiotics (8 isolates; MAR index of 0.56). The resistant pattern SXT-E-VA-TE, exhibited by 9 different *Escherichia coli* isolates and E-VA, also exhibited by 9 different *Escherichia coli* isolates were the commonest. Four isolates each exhibited a resistant pattern of SXT-E-C-VA-TE, SXT-E-VA, E-VA-TE and E-C-VA-TE. Of the 6 *Escherichia coli* isolates that were resistant to two different antibiotics, 2 each exhibited the pattern SXT-VA-TE, E-C-VA and SXT-C-VA. Among 80 isolates of *Escherichia coli* isolated from surface water and groundwater in dairy operations, 34 (42.5%) were resistant to one or more antibiotics and 22 (27.5%) were multi-antibiotic resistant (resistant to ≥ 3 antibiotics), with resistance to tetracycline, cefoxitin, amoxicillin/clavulanic acid, and ampicillin being the most common (Li *et al.*, 2014). Similarly to this work, we found that some of the isolates were resistant to ≥ 3 antibiotics. Multi-antibiotic resistant water *Escherichia coli* isolates have also been reported by Talukdar *et al.* (2013) and Subba *et al.* (2013).

Table 2: Antibiotic resistance profile and multiple antibiotic resistance index of individual *Escherichia coli* from different water sources in the Tamale Metropolis

<i>Escherichia coli</i> code	Source	Antibiotic resistant profile ^a	No. of antibiotics	MAR index
D3	Dam	C-VA-TE	3	0.33
RU2	Ruminant	E-C-CIP-VA-TE	5	0.56
P2	Poultry	E-CRO-VA-AMC	4	0.44
P1	Poultry	E-CRO-VA-CN	4	0.44
RU4	Ruminant	E-C-TE-AMC	4	0.44
P10	Poultry	E-C-VA	3	0.33
R8	Rain	E-C-VA	3	0.33
D7	Dam	E-C-VA-TE	4	0.44
T6	Tap	E-C-VA-TE	4	0.44
W5	Well	E-C-VA-TE	4	0.44
D1	Dam	E-VA	2	0.22
D2	Dam	E-VA	2	0.22
D4	Dam	E-VA	2	0.22
D5	Dam	E-VA	2	0.22
R3	Rain	E-VA	2	0.22
RU6	Ruminant	E-VA	2	0.22
T3	Tap	E-VA	2	0.22
T8	Tap	E-VA	2	0.22
W3	Well	E-VA	2	0.22
P3	Poultry	E-VA-TE	3	0.33
P9	Poultry	E-VA-TE	3	0.33
RU1	Ruminant	E-VA-TE	3	0.33
W10	Well	E-VA-TE	3	0.33
R4	Rain	E-VA-TE-AMC	4	0.44
R10	Rain	N/A	N/A	N/A
D8	Dam	SXT-C-VA	3	0.33
R5	Rain	SXT-C-VA	3	0.33
D9	Dam	SXT-C-VA-TE	4	0.44
T1	Tap	SXT-E-C	3	0.33
RU3	Ruminant	SXT-E-CRO-VA-TE	5	0.56
R9	Rain	SXT-E-C-VA	4	0.44
P5	Poultry	SXT-E-C-VA-TE	5	0.56
T4	Tap	SXT-E-C-VA-TE	5	0.56
T7	Tap	SXT-E-C-VA-TE	5	0.56
W7	Well	SXT-E-C-VA-TE	5	0.56
W2	Well	SXT-E-TE	3	0.33
D6	Dam	SXT-E-VA	3	0.33
P4	Poultry	SXT-E-VA	3	0.33
W4	Well	SXT-E-VA	3	0.33
W8	Well	SXT-E-VA	3	0.33
D10	Dam	SXT-E-VA-CN	4	0.44
P6	Poultry	SXT-E-VA-TE	4	0.44
P7	Poultry	SXT-E-VA-TE	4	0.44
R2	Rain	SXT-E-VA-TE	4	0.44
R7	Rain	SXT-E-VA-TE	4	0.44
RU5	Ruminant	SXT-E-VA-TE	4	0.44
T2	Tap	SXT-E-VA-TE	4	0.44
T5	Tap	SXT-E-VA-TE	4	0.44
T9	Tap	SXT-E-VA-TE	4	0.44
W1	Well	SXT-E-VA-TE	4	0.44
W9	Well	SXT-E-VA-TE	4	0.44
P8	Poultry	SXT-E-VA-TE-AMC	5	0.56
D11	Dam	SXT-E-VA-TE-CN	5	0.56
R6	Rain	SXT-VA-TE	3	0.33
W6	Well	SXT-VA-TE	3	0.33
R1	Rain	VA	1	0.11

^aAMC: Amoxicillin/clavulanic acid 30 µg, C: Chloramphenicol 30 µg, CIP: Ciprofloxacin 5 µg, CRO: Ceftriaxone 30 µg, CN: Gentamicin 10 µg, E: Erythromycin 15 µg, SXT: Suphamethoxazole/Trimethoprim 22 µg, TE: Tetracycline 30 µg, VA: Vancomycin 30 µg and N/A: Not applicable

Researches in the antibiotic susceptibility of *Escherichia coli* and other food/waterborne pathogens in Ghana are limited and the few researches available have concentrated more on human isolates (Adzitey, 2015). Saba and Tekpor (2015) in their work determined the antibiotic resistance patterns of *Escherichia coli* O157: H7 isolated from swimming pools and found that, the *Escherichia coli* were resistant to ceftriaxone (25%), chloramphenicol (21%), amoxicillin-clavulanic acid (14%), ciprofloxacin (11%) and gentamicin (4%). We found lower resistances to ceftriaxone (5.36%), amoxicillin-clavulanic acid (7.14%) and ciprofloxacin (1.79%) but higher for chloramphenicol (30.36%). Nonetheless, resistant to gentamicin was similar in both studies (4% versus 5.36%). Carnot *et al.* (2014) determined the antibiotic susceptibility of *Escherichia coli* isolated from in natural water and reported that 37.20 and 18.60% were resistant to tetracycline ciprofloxacin, respectively. Contrarily, we observed a higher resistance to tetracycline (57.14%) but a lower resistance to ciprofloxacin (1.79%). Of the 233 *Escherichia coli* isolated from household water supply tested against antibiotics, 45% were resistant to tetracycline, followed by 36% to trimethoprim-sulfamethoxazole, 17% to ciprofloxacin, 9% to ceftriaxone, 8% to chloramphenicol and 1% to gentamicin (Talukdar *et al.*, 2013). Comparable to this study, resistance to tetracycline, trimethoprim-sulfamethoxazole, chloramphenicol and gentamicin was lower but higher for ciprofloxacin and ceftriaxone.

Subba *et al.* (2013) reported that all *Escherichia coli* obtained from drinking water sources were susceptible to chloramphenicol, which was different in this study. Resistance of the *Escherichia coli* and thermotolerant *Escherichia coli* isolates to tetracycline was 93.5 and 100%, respectively (Subba *et al.*, 2013) which are higher as compared to this study. It has been established that antibiotic resistance patterns vary from one region to the other (Subba *et al.*, 2013; Talukdar *et al.*, 2013; Carnot *et al.*, 2014; Saba and Tekpor, 2015). These differences are as a result of variations in time and samples examined, sampling methodology employed and the extent to which antibiotics are used in the various regions (Adzitey, 2015). Antibiotic resistant *Escherichia coli* is potential source of transmission of resistance to other water borne pathogens. Transmission of resistant *Escherichia coli* through drinking water poses serious threats to humans and animals and consequently public health. In this study most of the *Escherichia coli* isolates were susceptible to ciprofloxacin (94.64%), gentamicin (91.07%) and ceftriaxone (89.29%). Therefore ciprofloxacin (flouoroquinolones), gentamicin (aminoglycosides) and ceftriaxone (cephalosporins) can be the first antibiotic of choice for treating *Escherichia coli* infection caused by the consumption of water in the Tamale Metropolis of Ghana.

In conclusion, *Escherichia coli* isolates from drinking water sources exhibited varying resistances to antibiotics. Averagely, 49.21% were susceptible, 12.90% were intermediate and 37.90% were resistant. High susceptibility was observed for ciprofloxacin, gentamicin and ceftriaxone while high resistances were observed for erythromycin and vancomycin. Multiple antibiotic index ranged from 0.11-0.56 (that is resistant to 1-5 different antibiotics). Majority of the *Escherichia coli* isolates were resistant to four antibiotics but the resistant pattern, SXT-E-VA-TE and E-VA were the commonest. It is important that the use of antibiotics by humans and in animal production be monitored to prevent the development of multi-resistant *Escherichia coli*. This study provides baseline information about the antibiotic susceptibility of drinking water *Escherichia coli* isolate in the Tamale Metropolis of Ghana.

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