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Prevalence and Antimicrobial Resistance Patterns of *E. coli* Isolates from Cow Milk, Milk Products and Handlers in the Tamale Metropolis of Ghana

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SUMMARY

This study was carried out in the Tamale Metropolis of Ghana to determine the prevalence and antibiotic resistance of *Escherichia coli* in pasteurized cow milk, cow milk products and hands of cow milk sellers. The conventional method and the disk diffusion method were used for the isolation and antibiotic susceptibility testing of *Escherichia coli*, respectively. The overall prevalence of *Escherichia coli* was 42.7% (128/300). Pasteurized milk and raw 'wagashie' were the most commonly contaminated (78.0%) source, followed by 'brukina' (54.0%), fried 'wagashie' (26.0%), left hand (12.0%) and right hand (8.0%). The prevalence of *Escherichia coli* in pasteurized milk and raw 'wagashie' was significantly higher ($p = 0.003$) than that of 'brukina', fried 'wagashie', left hand and right hand. Screening of 102 *Escherichia coli* isolates for their susceptible to antibiotics revealed that 49.0% were susceptible, 31.0% were resistant and 20.0% were intermediate to the various antibiotics post testing. Resistance to ampicillin (65.7%) was the highest, followed by erythromycin (61.8%). The *Escherichia coli* isolates were generally susceptible to ciprofloxacin (88.2%) and gentamicin (71.6%). The *Escherichia coli* isolates also exhibited 40 antibiotic resistance patterns with the pattern E (erythromycin) being the commonest. Twenty-nine 29 (28.4%) were resistant to three different classes of antibiotics, 20 (19.6%) were resistant to four different classes of antibiotics, 5 (4.9%) were resistant to five different classes of antibiotics and 1 (1.0%) was resistant to six different classes of antibiotics. The study revealed that milk, its products and handlers in the Tamale metropolis were contaminated with *Escherichia coli* which are resistant to a number of antibiotics.

Key words: Antibiotics, *Escherichia coli*, hands, milk, milk products, sellers.

INTRODUCTION

Milk is an opaque white fluid produced by the mammary glands of mammals. It has been part of human diet for years and is valued as a natural and

traditional food. Milk and milk products are important sources of good quality protein, carbohydrate, fat, the B vitamins (B2 and B12) and minerals (iodine, potassium and phosphorus) (Lucey, 2015). Milk also contains dissolved gasses (5% by volume), mainly; carbon dioxide (CO_2), nitrogen (N) and oxygen (O_2) (Lucey, 2015).

Though milk and milk products provide a wealth of nutritional benefits, raw or processed milk is a well-known good medium that harbours microorganisms such as *Salmonella* species, *Escherichia coli* and *Listeria* species that can pose serious health risks especially to people with weak immune systems, older adults, pregnant women, and children (Murinda *et al.*, 2004; Oliver *et al*., 2005; United States Food and Drug Administration, 2012). *Escherichia coli* are widely distributed in the intestine of humans and warm-blooded animals. They are the predominant facultative anaerobes in the bowel and part of the essential intestinal flora that maintains the physiology of the healthy host (Feng *et al.,* 2002). Some *Escherichia coli* strains are known to be pathogenic bacteria, causing severe intestinal and extra intestinal diseases in man (Kaper *et al.*, 2004).

The development of antibiotic resistance by microorganisms has been linked to the use and misuse of antibiotics for therapeutic purposes and for growth promotion (Forshell and Wierup, 2006; Adzitey, 2015a). Antibiotic resistance is a global problem, but demand for antibiotics continues to rise, particularly to treat patients suffering from bacterial infections (Liu *et al.*, 2015). In Ghana, milk and milk products are cherished by many. Raw and/or pasteurised cow milk are normally purchased from cattle farmers especially the Fulani herdsmen and are used in the preparation of various milk products including 'brukina' or 'fula' and 'wagashie'. The manner in which cow milk, pasteurised milk, 'brukina' and 'wagashie' are processed expose them to contamination by microorganisms. However, not much is known about the rate of contamination and antimicrobial susceptibility of microorganisms of milk origin in the Tamale Metropolis of Ghana. There is therefore the need to determine the prevalence and antibiotic resistance of *Escherichia coli* isolates from milk, milk products and handlers in the study area. This will help create awareness of the safety of milk and milk products in the Tamale Metropolis.

MATERIALS AND METHODS

Study Area

This study was conducted in the Tamale metropolis (Figure 1). Tamale is the capital town of the Northern region of Ghana. The Metropolis has a total estimated land size of 646.90180sqkm (Ghana Statistical Service, (GSS), 2010). Geographically, the Metropolis lies between latitude 9º16 and 9º 34 North and longitudes 0º 36 and 0º 57 West (GSS, 2010). It covers an area of 930km with a population of about 300,000 and a growth rate of 2.5% (GSS, 2010). Tamale has annual rainfall of 1100mm and mean temperature ranges of 28°C to 43°C (GSS, 2010).

Figure 1: Map of Tamale Metropolis

Samples Examined

Three hundred samples were randomly collected from milk and milk product sellers at various markets in the Tamale metropolis. The samples collected were; pasteurized cow milk, raw 'wagashie', fried 'wagashie', 'brukina', right and left palm swabs. Fifty samples of each of them were collected. Milk and milk products were collected in sterile polythene bags whilst sterile swabs were used to swab the hands of milk and milk product sellers. The sellers kept the samples in the sterile bags as they sell to their customers without the researchers touching the samples. Observations were also made during sample collection. The samples collected were kept in an ice chest containing ice block and transported to the

Laboratory (Spanish Laboratory, Nyankpala Campus). Microbial analyses were carried out immediately upon arrival at the laboratory.

Isolation of *Escherichia coli*

Isolation of *Escherichia coli* was done using a modified method of the USA Food and Drug Administration-Bacteriological Analysis Manual (FDA-BAM). Briefly, pasteurized cow milk, 'brukina', 'wagashie', and hand swabs were dispersed or placed in buffered peptone water and incubated at 37 ℃ for 24 hours under aerobic conditions. The proportions of the samples to buffered peptone water were: 10ml of pasteurized milk sample: 90ml of buffered peptone water, 10ml of 'brukina': 90ml of buffered peptone water, 10g of 'wagashie' (fried and raw): 90ml of buffered peptone water, and a swab of the entire surface of the palm of hand (left and right): 10ml of buffered peptone. After incubation, a loopful of the culture from the buffered peptone water was streaked on Levine eosin-methylene blue (LEMB) agar and then incubated at 37 ℃ for 24 hours under aerobic condition. Presumptive *E. coli* colonies on the LEMB agar were red- pink with or without metallic sheen. Such isolates were streaked onto nutrient agar and incubated for 24 hours under aerobic condition to obtain pure cultures. Pure cultures were identified and confirmed using Gram stain, growth on brilliant green bile and latex agglutination test for *E. coli*. All media used were purchased from Oxoid Limited, Basingstoke, UK.

Antibiotic Sensitivity Test

The disk diffusion method of Bauer *et al*. (1966) was used to determine the antibiotic resistance of 102 *Escherichia coli* isolates. Single colony of pure cultures was picked and grown in tryptic soy broth (TSB). It was incubated at 37°C for 16hrs and the concentration adjusted using sterile TSB until 0.5Macfarland turbidity was attained. Approximately, 100µl of the culture was swabbed unto Mueller Hinton agar using a sterile cotton swab. Antimicrobial disks (chloramphenicol, C (30µg), sulfamethoxazole-trimethoprim, SXT (1.25-23.75µg), tetracycline, Te (30µg), amoxycillin, Amc (10µg), erythromycin, E (15µg), ciprofloxacin, Cip (5µg), ceftriaxone, Cro (30µg) and gentamicin, Cn (10µg)) were placed on the surface of the Mueller Hinton agar plates at a distance to avoid overlapping of inhibition zones. The plates were incubated at 37°C for 16-18 hours and the results were interpreted as Susceptible (S), Intermediate (I) and Resistance (R) according to the Clinical Laboratory Standard Institute (2008). All media and antibiotic disks used were purchased from Oxoid Limited, Basingstoke, UK.

Statistical Analysis

The data obtained was analysed 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

Prevalence of *Escherichia coli* in Cow Milk, Cow Milk Products and Handler's Hands

The prevalence of the *Escherichia coli* in the pasteurized cow milk, 'wagashie' (raw and fried), hands of cow milk sellers (left and right hands) and 'brukina' analysed in the Tamale metropolis of Ghana is shown in Table I. Out of the 300 samples examined, 128 (42.7%) were positive for *Escherichia coli*. The distribution of *Escherichia coli* was 78.0% (39/50), 78.0% (39/50), 54.0% (27/50), 26.0% (13/50), 12.0% (6/50) and 8.0% (4/50) for pasteurized milk, raw 'wagashie', 'brukina', fried 'wagashie', left hand and right hand, respectively. The prevalence of *Escherichia coli* in pasteurized milk and raw 'wagashie' was significantly higher ($p = 0.003$) than the rests of the samples examined. The prevalence of *Escherichia coli* in 'brukina' was also significantly higher ($p = 0.003$) than that of fried 'wagashie', left and right hand samples. Furthermore, the prevalence of *Escherichia coli* in fried wagashie was statistically higher ($p = 0.022$) than that of right hand but not left hand samples; right hand and left hand positive samples did not differ statistically ($p = 0.600$) from each other.

Table I: Distribution of *Escherichia coli* in milk and its related samples

Type of sample	Number of samples tested	Number of samples positive	Percentage prevalence
Raw 'wagashie'	50	39	78.0
Pasteurized cow milk	50	39	78.0
'Brukina'	50	27	54.0
Fried 'wagashie'	50	13	26.0
Left hand	50	6	12.0
Right hand	50	4	8.0
Total	300	128	42.7

Antibiotic Susceptibility of *Escherichia coli* in Cow Milk, Cow Milk Products and Handlers

The antibiotic resistance of the *Escherichia coli* isolates is shown in Table II. Of the 102 *Escherichia coli* isolates subjected to antibiotic susceptibility testing, 49% (399/816) were susceptible, 31% (257/816) were resistant and 20% (160/816) were intermediate resistant to all the antibiotics. A high percentage of the *Escherichia coli* isolates were resistant to ampicillin (65.7%) and erythromycin (61.8%). Susceptibility to antibiotics was highest for ciprofloxacin (88.2%), followed by gentamicin (71.6%), suphamethoxazole/trimethoprim (58.8%) and chloramphenicol (58.8%), and ceftriaxone (54.9%). The *Escherichia coli* isolates also exhibited intermediate resistances to all the antibiotics. The antibiotic resistant patterns revealed that 22 isolates were resistant to only one antibiotic, 19 isolates were resistant to two antibiotics, 29 isolates were resistant to three antibiotics, 20 isolates were resistant to four antibiotics, 5 isolates were resistant to five antibiotics and 1 isolate was resistant to six antibiotics (Table III). Six *Escherichia coli* isolates were resistant to none of the antibiotics. Resistance to only erythromycin was the commonest pattern.

Table II: Percentage antibiotic resistance of *E. coli* isolated from milk and its related samples

Antimicrobial	*n/102	R (%)	I (%)	S (%)
Ampicillin (Amp) 30 µg	67	65.7	18.6	15.7
Chloramphenicol (C) 30 µg	18	17.6	23.5	58.8
Ciprofloxacin (Cip) 5 µg	2	1.96	9.8	88.2
Ceftriaxone (Cro) 30 µg	35	34.3	10.8	54.9
Gentamicin (Cn) 10 µg	3	2.9	25.5	71.6
Erythromycin (E) 15 µg	63	61.8	33.3	4.90
Suphamethoxazole/trimethoprim (Sxt) 22 µg	22	21.6	19.6	58.8
Tetracycline (Te) 30 µg	47	46.1	15.7	38.2

*n, number of resistant *Escherichia coli*; S, susceptible; I, Intermediate; R, resistant

DISCUSSION

Prevalence of *Escherichia coli* in Milk and Its Related Samples The aim of this study was to determine the prevalence and antibiotic resistance of *Escherichia coli* isolates from pasteurized cow milk, cow milk products and hand samples of cow milk sellers. The overall prevalence of *Escherichia coli* in the milk and its related samples was 42.7%. Pasteurized milk and raw 'wagashie' were the frequently most contaminated source, followed by 'brukina', fried 'wagashie', left hand and right hand. The prevalence of *Escherichia coli* in pasteurized milk and raw 'wagashie' was statistically higher ($p = 0.003$) than the other samples. The prevalence of *Escherichia coli* in 'brukina' was also statistically higher ($p = 0.003$) than that of fried 'wagashie', left and right hand samples. Left and right hand samples did not differ statistically ($p = 0.600$) from each other. The high contamination of pasteurized milk, raw 'wagashie' and 'brukina' is as a result of the unhygienic practices of sellers of milk and milk products in the Tamale metropolis engaged in. It was observed during sampling that most milk and milk products were sold in the open market in containers that were not always covered. Houseflies were seen hovering around these containers and there were instances that houseflies were found in some of the milk being sold. The containers used for selling the milk and milk products were not always clean and were washed with water at room temperature without detergent. This same water was also used throughout the selling period without changing it. Some milk and milk product seller's worn unclean clothes, use dirty/ uncleaned hands to sell milk and allowed other people

to sell milk to consumers. Raw 'wagashie' were placed in unclean water and after frying they are left on flat open pans for sale, thus exposing them again to the environment for contamination. It has been shown that contamination of raw milk could originate from surrounding environment especially during milking and milk handling, from water and milking equipment and facilities (Oliver *et al.,* 2005). Similarly, to this study, Adzitey *et al*. (2016) found that the prevalence of *Escherichia coli* in milk samples was significantly higher (P<0.05) than hand samples in the Nyankpala Community of Ghana. Saba *et al*. (2015) also found that 74 (49.3%) of the raw cow milk samples collected from Tamale Metropolis were contaminated with *E. coli* and other enterobacteria. *Escherichia coli* has also been previously isolated from the environment (e.g. drinking water, faeces or beef samples) where this study was carried (Adzitey *et al.,* 2015a; 2015b; 2015b; 2016; Saba *et al*., 2015).

The presence of *Escherichia coli* in milk and milk products have also been reported by other researchers in different countries. In Nigeria, the prevalence of *Escherichia coli* in raw milk, yoghurt and cheese were reported to be 9.2%, 29.5% and 61.3%, respectively (Abike *et al.,* 2015). In India,rates of 20.0%, 15.0%, 15.0%, 10.0%, and 8.0% for curd, 'khoa', raw milk, packed milk, and 'paneer', respectively were reported (Khan *et al.,* 2014). The prevalence of *Escherichia coli* in 75 raw milk collected from small holder dairy farmers, street vendors and outlet shops in Arusha and Arumeru Districts in Tanzania was 90.67% (Lubote *et al.,* 2014). A prevalence range of 0%-5.7% (in Europe), 0%-3.8% (in United States), and 0.3% (in New Zealand) for human pathogenic *Escherichia coli* has been reported in raw milk samples (Griffiths, 2010; Soboleva, 2014; Robinson *et al.*, 2014). In this present study, the prevalence of *Escherichia coli* in the milk and milk samples ranged from 8.0% to 78.0%. Griffiths (2010) also reported that, the prevalence of pathogens in milk is influenced by factors like farm size, farm management practices, milking facilities, season, number of animals on farm and hygiene.

Table III: Antibiotic resistant pattern of *Escherichia coli* isolates

Number of antimicrobial resistance	Antimicrobial resistance pattern (Number of isolates)	Pattern number(s)	Number of isolates (%)
Zero antibiotics	-		6 (5.9)
One	AMP (7)	1	
	CRO (2)	2	22 (21.6)
	E (12)	3	
	TE (1)	4	
Two	AMP, CRO (2)	5	19 (18.6)
	AMP, TE (2)	6	
	C, CRO (2)	7	
	C, E (2)	8	
	E, AMP (6)	9	
	E, CRO (1)	10	
	E, TE (2)	11	
	SXT, AMP (1)	12	
	TE, CRO (1)	13	
Three	AMP, TE, CRO (5)	14	
	C, E, AMP (1)	15	
	C, TE, CRO (1)	16	
	E, AMP, CRO (5)	17	
	E, AMP, TE (9)	18	29 (28.4)
	E, TE, CRO (2)	19	
	SXT, AMP, TE (2)	20	
	SXT, C, AMP (1)	21	
	SXT, E, AMP (2)	22	
	SXT, E, TE (1)	23	
Four	C, AMP, TE, CRO (2)	24	
	C, E, AMP, CRO (2)	25	
	C, E, AMP, TE (1)	26	
	C, E, TE, CRO (1)	27	
	CIP, TE, CRO, CN (1)	28	
	E, AMP, TE, CRO (2)	29	
	E, CIP, AMP, TE (1)	30	
	E, TE, CRO, CN (1)	31	
	SXT, AMP, TE, CN (1)	32	
	SXT, C, E, AMP (1)	33	
	SXT, E, AMP, CRO (2)	34	
	SXT, E, AMP, TE (5)	35	20 (19.6)
Five	SXT, AMP, TE, CRO, CN (1)	36	
	SXT, C, AMP, TE, CRO (1)	37	
	SXT, C, E, AMP, TE (2)	38	5 (4.9)
	SXT, E, AMP, TE, CRO (1)	39	
Six	SXT, C, E, AMP, TE, CRO (1)	40	1 (1.0)

Antimicrobial Susceptibility

The emergence of multidrug resistant pathogens is of global concern and has instigated several investigations and researches. The U.S. Centres for Disease Control and Prevention (CDC) estimated that antibiotic resistance is responsible for more than 2 million infections and 23,000 deaths each year in the United States (CDC, 2013). In Europe, an estimated 25,000 deaths are attributable to antibiotic-resistant infections (EMA and ECDC, 2009). Although reliable estimates of losses in the developing world are not

available, it is estimated that 58,000 neonatal sepsis deaths are attributable to drug- resistant infections in India alone (Laxminarayan *et al.,* 2013). This study revealed that *Escherichia coli* isolates of milk products and handlers exhibited 49%, 31% and 20% susceptibility, resistance and intermediate resistance, respectively. Adzitey *et al.* (2016) reported an overall resistance, intermediate resistance and susceptibility of 14.35% 21.30% and 64.35%, respectively for *Escherichia coli* isolated from milk and hands of milkers. Thus the overall resistance has increased compared to previous studies. A study by Khan *et al.* (2014), involving *Escherichia coli* isolated from milk and milk products revealed an overall resistance of 85.0% and susceptibility of 15.0%. Resistances greater than 60.0% occurred for ampicillin and erythromycin in this study. Furthermore, susceptibility greater than 70% occurred for ciprofloxacin and gentamicin. The *Escherichia coli* isolates also exhibited intermediate resistances to all the antibiotics. Intermediate resistances were relatively high for chloramphenicol (23.5%), gentamicin (25.5%) and erythromycin (33.3%). Isolates that exhibited intermediate resistances have a higher tendency of becoming resistant (Adzitey *et al.,* 2012). Abike *et al.* (2015), found that *Escherichia coli* isolated from milk and milk products were resistant to gentamicin (6.8%) and tetracycline (56.8%), however, lower resistances to gentamicin and tetracycline was observed in this study. Khan *et al.* (2014), found higher resistances to ampicillin (98.0%), ceftriaxone (95.0%), chloramphenicol (95.0%), tetracycline (92.0%) and gentamicin (80.0%) as compared to the current study.

Escherichia coli isolates that are multi-drug resistant are those that are resistant to 3 or more different classes of antibiotics. From Table III, 28.4% of the *Escherichia coli* isolates were resistant to three antibiotics, 19.6% of the isolates were resistant to four antibiotics, 4.9% of the isolates were resistant to five antibiotics and 1% of the isolate was resistant to six antibiotics. The 102 milk and milk product *Escherichia coli* isolates exhibited 40 different antibiotic resistant patterns. Resistant to erythromycin alone was the commonest (exhibited by 12 isolates). The resistance pattern erythromycin-ampicillin-tetracycline (exhibited by 9 isolates) was the commonest multidrug resistant pattern. This was followed by the resistant patterns ampicillin-tetracycline-ceftriaxone, erythromycin-ampicillin-ceftriaxone and suphamethoxazole/trimethoprim-erythromycin-ampicillin-tetracycline (exhibited by 5 isolates). One *Escherichia coli* isolate was resistant to 6 different antibiotics, thus suphamethoxazole/trimethoprim-chloramphenicol-erythromycin-ampicillin-tetracycline-ceftriaxone.

Multidrug resistant milk and milk products *Escherichia coli* isolates have been reported by other researchers. Abike *et al.* (2015) reported that, 51.4% of *Escherichia coli* isolates were resistant to three antibiotics, 34.2% were resistant to four antibiotics and 17.1% were resistant to five antibiotics. Magiorakos *et al.* (2012) reported that, bacteria that resist multiple antibiotics are most threatening to public health. Bacterial acquire resistance through spontaneous mutation or by DNA transfer (Coates *et al.*, 2002) which is driven by the indiscriminate use of antibiotics for therapeutic and growth purposes in humans and animals (Forshell and Wierup, 2006).

CONCLUSION

The overall prevalence of *Escherichia coli* species was 42.67%. Pasteurized milk and raw 'wagashie' were the most contaminated sources, followed by 'brukina', fried 'wagashie', left hand and right hand. It is therefore important that milk handlers, processors and sellers in Tamale and Ghana at large observe hygienic practices in the handling, processing and selling of raw milk and milk products. *Escherichia coli* isolates of milk, milk products and handlers exhibited varying percentages of susceptibility, resistance and intermediate resistance. Higher susceptibility occurred for chloramphenicol, ciprofloxacin, ceftriaxone, gentamicin and suphamethoxazole/trimethoprim, while higher resistance occurred for ampicillin, erythromycin and tetracycline. Majority of the *Escherichia coli* isolates were resistant to three antibiotics but resistance to erythromycin was the commonest.

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