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Research Article
Antibiotic Susceptibility of *Escherichia coli* Isolated from Milk and Hands of Milkers in Nyankpala Community of Ghana

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Abstract

Background and Objective: The use of antibiotics in animal production is a major concern to health providers and consumers. This study was carried out to determine the prevalence of resistant *Escherichia coli* in cow milk and hands of milkers. **Materials and Methods:** A total of 104 cow milk and hands of milkers were examined for the presence of *Escherichia coli.* Isolation of *Escherichia coli* was done using the convention method in the US Food and Drug Administration-Bacteriological Analysis Manual (FDA-BAM). Antibiotic susceptibility test was done using the disc diffusion method and the results interpreted using the clinical and laboratory standards institute guidelines. Prevalence data was analyzed using SPSS version 17. **Results:** The overall prevalence of *Escherichia coli*in the milk and hand samples was 40.38%. The prevalence of *Escherichia coli* in milk collected directly from the udder, in milk collected from milking containers, right hand swabs and left hand swabs were 61.54, 57.69, 23.08 and 19.23%, respectively. The prevalence of *Escherichia coli* in milk samples was significantly higher ($p<0.05$) than hand samples. Twenty seven *Escherichia coli* isolated from the milk and hand samples were screened against 8 different antibiotics. Overall, 14.35% of the *Escherichia coli* isolates were resistant, 21.30% were intermediate and 64.35% were susceptible. Resistance to ceftriaxone (29.63%) was the highest, followed by tetracycline (25.93%) and ampicillin (22.22%). A relatively higher percentage of the isolates exhibited intermediate resistance to ampicillin (51.85%), erythromycin (48.15%) and chloramphenicol (37.04%). *Escherichia coli* isolates also exhibited 13 antibiotic resistant patterns. Five isolates were resistant to 3 or more different classes of antibiotics. **Conclusion:** This study revealed that *Escherichia coli* from cow milk and hands of milkers in the Nyankpala community are resistant to some antibiotics. Consumers are expose to *Escherichia coli* infection from drinking of milk produced in the Nyankpala community of Ghana.

Key words: Antibiotics, *Escherichia coli*, hand, milk, resistance

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Escherichia coli are Gram-negative, oxidase-negative, catalase-positive, non-spore forming and facultative anaerobic bacteria naturally found in the gastrointestinal tract of animals and the environment through cross contamination (Aarestrup *et al*., 2008; Adzitey *et al*., 2014; CDC., 2014a). *Escherichia coli* cells are rod-shaped with about 2.0 µm long and 0.25-1.0 µm in diameter (Kubitschek, 1990). They also ferment glucose or lactose and are members of the Enterobacteriaceae family (CDC., 2014a). Most *Escherichia coli* strains are harmless and form part of the normal flora of the gastrointestinal tract of animals (Aarestrup *et al*., 2008). However, pathogenic *Escherichia coli* cause vomiting, abdominal crumps, diarrhoea, little or no fever, chills, a generalized malaise, hemorrhagic colitis and haemolytic uremic syndrome (CDC., 2014a; Feng *et al*., 2002; Shahina *et al*., 2011). *Escherichia coli* have been isolated from a number of water and food sources and products of animal origin (Goja, 2013; Reuben and Owuna, 2013; Bonyadian *et al*., 2014; Geck *et al*., 2014; Adzitey, 2015a; Abike *et al*., 2015; Anachinaba *et al*., 2015; Adzitey *et al*., 2015a; Adzitey *et al*., 2015b; Hassan *et al*., 2015; Saba *et al*., 2015; Tersagh *et al*., 2015; Kurnia *et al*., 2015) and have been implicated in some foodborne outbreaks (CDC., 2014b; CDC., 2015). For instance CDC (2014b) reported an outbreak of Shiga toxin-producing *Escherichia coli* O157:H7 (STEC O157:H7) infections resulting from the consumption of contaminated ground beef. Twelve people were infected and 58.0% of them were hospitalized (CDC., 2014b). An outbreak of *Escherichia coli* from the consumption of rotisserie chicken salad caused 5 ill people to be hospitalized and 2 developed hemolytic uremic syndrome (CDC., 2015).

Antibiotics are very important for the treatment of bacterial infections in humans and animals (Adzitey, 2015b). Despite this, concerns about their usage continue to receive major attention and debates among stakeholders. This is because their usage has been associated with the increasing and unprecedented multidrug resistant patterns exhibited by bacteria (Aarestrup *et al*., 2008). The FDA (2014) reported that resistance in *Escherichia coli* is consistently the highest for antimicrobial agents that have long been in use in human and veterinary medicine. *Escherichia coli* isolates of animal origin have been demonstrated to be resistant to ampicillin, chloramphenicol, ciprofloxacin, gentamicin, erythromycin, tetracycline and many more antibiotics (Reuben and Owuna, 2013; Bonyadian *et al*., 2014; Abike *et al*., 2015; Adzitey, 2015b; Adzitey *et al*., 2013).

Investigations on the resistant patterns of *Escherichia coli* in foods and products of animal origin are limited in Ghana and data on such information is unavailable in the Nyankpala community of Ghana. To the best of our knowledge, this study reports for the first time on the prevalence and antibiotic susceptibility of *Escherichia coli* isolated from cow milk and hands of milkers in the Nyankpala community of Ghana. It also provides a database on the prevalence of resistant *Escherichia coli* in cow milk and hands of milkers based on which other studies can be compared to monitor changes in the resistant patterns of this bacterium in Nyankpala community and Ghana as a whole.

MATERIALS AND METHODS

Collection of samples: The study was carried out in the Nyankpala community of the Northern region of Ghana. One hundred and four samples made up of cow milk and hands of milkers were randomly collected from cattle farmers within the Nyankpala community. The samples were milk collected directly from the udder (n = 26), milk collected from milking containers (n = 26), right hand swab of milkers (n = 26) and left hand swab of milkers (n = 26). The study was conducted within the period of July-December, 2015.

Isolation and identification of *Escherichia coli*: Bacteriological analysis of *Escherichia coli* was done using a slightly modified procedure in the US Food and Drug Administration-Bacteriological Analysis Manual (FDA-BAM). For milk samples, 10 mL were enriched in 90 mL buffered peptone water (Oxoid, Basingstoke, UK), while for swab samples, 1 swab was enriched in 9 mL buffered peptone water (Oxoid, Basingstoke, UK). Samples in buffered peptone water were incubated at 37°C for 24 h under aerobic conditions. After which a loopful of the enriched culture was streaked onto LEMB agar (Oxoid, Basingstoke, UK) and incubated at 37°C for 24 h under aerobic condition. Presumptive *Escherichia coli* colonies on LEMB agar appear as dark centered and flat with or without metallic sheen. Presumptive *Escherichia coli* were purified on nutrient agar (Oxoid, Basingstoke, UK) and pure cultures were identified and/or confirmed using Gram staining, biochemical tests (indole production, utilization of citrate, lactose production) and *Escherichia coli* latex agglutination test.

Antimicrobial susceptibility of *Escherichia coli*: The disk diffusion method of Bauer *et al*. (1966) was used to determine the antibiotic resistance of 27 isolated strains of *Escherichia coli* against the following antibiotics; ampicillin (Amp) 30 µg, chloramphenicol (C) 30 µg, ciprofloxacin (Cip) 5 µg, ceftriaxone (Cro) 30 µg, gentamicin (Cn) 10 µg, erythromycin (E) 15 µg, suphamethoxazole/trimethoprim (Sxt) 22 µg and

tetracycline (Te) 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 microliters of the culture was then spread plated unto Mueller Hinton agar (Oxoid, Basingstoke, UK) using a sterile cotton swab. Four 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 resistant according to clinical and laboratory standards institute guidelines (CLSI., 2006).

Statistical analysis: Prevalence data was analysed using generalized linear model of Statistical Package for the Social Sciences (SPSS) version 17.

RESULTS AND DISCUSSION

Distribution of *Escherichia coli* in cow milk and hands of milkers: The distribution of *Escherichia coli* in cow milk and hands of milkers in the Nyankpala community of Ghana is presented in Table 1. From Table 1, *Escherichia coli* were isolated from milk collected directly from the udder (16/26), milk collected from milking containers (15/26), right hand (6/26) and left hand (5/26) samples. The prevalence of *Escherichia coli* in milk samples was significantly higher ($p<0.05$) than that of hand samples. Therefore, milk samples collected directly from the udder was most contaminated by *Escherichia coli*, followed by milk samples collected from milking containers, right hand and left hand. In addition milk samples were highly contaminated by *Escherichia coli* than hand samples. Milk is a rich source of various nutrients. Teller (2012) stated that the purpose of milk in nature is to provide all the nutrients to sustain health, grow, protect and heal. The nutrients in milk serve as an ideal medium for the growth of microorganisms. The primary source of *Escherichia coli* contamination in this study can be faecal contamination. It was observed during data collection that some of the udders of the lactating cows had faeces on them. These udders were not disinfected thoroughly before milking. The udder of these lactating cows could have been contaminated by faeces as a result of the animal lying on dirty litters in the farms visited. Furthermore, milk samples collected from milking containers may have been contaminated by milk collected from the udder which was kept in them or from the unsterilized containers used for collecting milk. Hands may have been contaminated by faeces on the udder during milking or from the hands of the milkers due to lack of proper personal hygiene.

The presence of *Escherichia coli* in milk and its related samples have been reported by other researchers. Bonyadian *et al*. (2014) examined 200 raw milk samples and found that 48% (96) were contaminated by *Escherichia coli*. They also found that 48% (24/50) of unpasteurized cheese samples were contaminated by *Escherichia coli*. Reuben and Owuna (2013) reported that 4.5% (19/420) of locally fermented milk samples were contaminated by *Escherichia coli* O157:H7. The prevalence of *Escherichia coli* were 9.2, 29.5 and 61.3% in raw milk, yoghurt and cheese, respectively (Abike *et al*., 2015). The prevalence of *Escherichia coli* in milk samples in this current study was higher than that reported by Reuben and Owuna (2013), Bonyadian et al. (2014) and Abike *et al*. (2015).

Antibiotic resistance of *Escherichia coli* strains: The antimicrobial resistance test of the 27 *Escherichia coli* determined against 8 antimicrobial agents is shown in Table 2. The overall resistance, intermediate and susceptibility were 14.35% (31/216), 21.30% (46/216) and 64.35% (139/216), respectively. Resistant to ampicillin (22.22%), ceftriaxone (29.63%) and tetracycline (25.93%) was relatively high compared to the other antibiotics examined. Higher intermediate resistances occurred for ampicillin (51.85%), chloramphenicol (37.04%) and erythromycin (48.15%).

Table 1: Distribution of *Escherichia coli* in milk and hands of milkers

	No. of samples		
Sources	Tested	Positive	Percentage prevalence
Milk directly from udder	26	16	61.54
Milking containers	26	15	57.69
Left hand	26	5	19.23
Right hand	26	6	23.08
Total	104	42	40.38

Table 2: Percentage antibiotic resistance of *Escherichia coli* in milk and hands of milkers

	Escherichia coli			
Antimicrobial	*n/27	R (%)	I (%)	S (%)
Ampicillin (Amp) 30 µg	6	22.22	51.85	25.93
Chloramphenicol (C) 30 µg	4	14.81	37.04	48.15
Ciprofloxacin (Cip) 5 µg	0	0.00	0.00	100.00
Ceftriaxone (Cro) 30 µg	8	29.63	7.41	62.96
Gentamicin (Cn) 10 µg	3	11.11	14.81	74.07
Erythromycin (E) 15 µg	3	11.11	48.15	40.74
Suphamethoxazole/trimethoprim (Sxt) 22 µg	0	0.00	7.41	92.59
Tetracycline (Te) 30 µg	7	25.93	3.70	70.37

*n: No. of resistant *Escherichia coli*, S: Susceptible, I: Intermediate and R: Resistant

Intermediate resistance refers to those *Escherichia coli* species that were not clearly resistant or susceptible (Adzitey, 2015b). It has been suggested in clinical diagnoses that patients with intermediate results can be given a higher dosage of antibiotics (Lorian, 2005). Organisms that exhibit intermediate resistance also have the tendency to easily become resistant (Adzitey *et al*., 2012). All the isolates were susceptible to ciprofloxacin (100.00%). A high percentage of the *Escherichia coli* were also susceptible to suphamethoxazole/trimethoprim (92.59%), gentamicin (74.07%), tetracycline (70.37%) and ceftriaxone (62.96%). Other researchers have also reported the sensitivity of *Escherichia coli* from milk and milk products to different antibiotics. Abike *et al*. (2015) reported that *Escherichia coli* isolated from raw cow milk, yoghurt and cheese were resistant to gentamicin (6.81%) and tetracycline (56.80%), which were higher than that observed in this study. *Escherichia coli* isolated from raw cow milk were resistant to ampicillin (34.00%), gentamicin (30.55%), trimethoprim/sulfamethoxazole (22.22%) and ciprofloxacin (16.66%) (Bonyadian *et al*., 2014). Comparatively, this study found lower resistances to ampicillin and gentamicin. No resistances were found for trimethoprim/sulfamethoxazole and ciprofloxacin. Reuben and Owuna (2013) found that *Escherichia coli* isolated from Nigerian fermented milk samples were resistant to tetracycline (100.00%), erythromycin (94.70%), sulphamethoxazole/trimethoprim (84.20%) and chloramphenicol (68.40%). No or lower resistances to these antibiotics was found.

The antibiotic resistance patterns are shown in Table 3. *Escherichia coli* exhibited 13 antibiotic resistant patterns. Resistance to 3 different classes of antibiotics were exhibited by 2 *Escherichia coli* isolates. Resistances to 4 different classes of antibiotics were exhibited by 3 *Escherichia coli* isolates. Thus the following multidrug resistances were found; ampicillin-gentamicin-erythromycin (AmpCnE), gentamicin-ceftriaxone-tetracycline (CnCroTe), ampicillin-chloramphenicol-ceftriaxone-erythromycin (AmpCCroE), ampicillin-chloramphenicol-ceftriaxone-tetracycline (AmpCCroTe) and chloramphenicol-gentamicin-ceftriaxone-erythromycin (CCnCroE). The use of antibiotics in animal production and the treatment of humans have been attributed to the increasing unprecedented multidrug exhibited by microorganisms (Aarestrup *et al*., 2008; Lorian, 2005; Tadesse *et al*., 2012). A wide variety of antibiotics have been suggested to be used in the treatment of infections caused by *Escherichia coli* (Zhao *et al*., 2001). Pitout (2012) reported that the β-lactams, fluoroquinolones, aminoglycosides and trimethoprim-sulfamethoxazole are often used to treat community and hospital infections due to *Escherichia coli*. Also in the present study, all the *Escherichia coli* isolates were susceptible to ciprofloxacin (100.00%), therefore, ciprofloxacin (flouroquinolones) can be the first drug of choice for treating *Escherichia coli* infection caused by the consumption of milk in the Nyankpala community of Ghana. In the absence of ciprofloxacin, suphamethoxazole/trimethoprim may be used before considering gentamicin and tetracycline.

Table 3: Antibiotic resistance profile *Escherichia coli* isolated from milk and hands of milkers

Sources	Antibiotic resistant pattern[a]	No. of antibiotics
Milk container	Amp	1
Milk container	AmpCCroE	4
Right hand	AmpCCroTe	4
Left hand	AmpCnE	3
Milk container	AmpE	2
Right hand	AmpTe	2
Milk directly from udder	C	1
Left hand	CCnCroE	4
Milk directly from udder	CnCroTe	3
Left hand	Cro	1
Milk container	CroTe	2
Milk container	CroTe	2
Milk container	CroTe	2
Milk directly from udder	E	1
Left hand	E	1
Right hand	E	1
Milk container	E	1
Milk container	E	1
Milk container	E	1
Milk directly from udder	ETe	2

[a]Amp: Ampicillin 30 μg, C: Chloramphenicol 30 μg, Cro: Ceftriaxone 30 μg, Cn: Gentamicin 10 μg, E: Erythromycin 15 μg, Te: Tetracycline 30 μg and N/B seven *Escherichia coli* isolates were not resistant to any of the antibiotics

CONCLUSION

This study revealed that milk and its related samples are contaminated with *Escherichia coli* and some of these bacteria are resistant to antibiotics. Consumers of milk in the Nyankpala community of Ghana are at risk of *Escherichia coli* infection and transfer of resistant *Escherichia coli* species. The hygiene employed in handling milk in the community should be improved to ensure that consumers are served with *Escherichia coli* free milk.

SIGNIFICANCE STATEMENT

Antibiotic resistance of foodborne pathogens is a concern worldwide. This is the first report on the prevalence of resistant *E. coli* in milk and its related sample in Nyankpala. Significantly, this study provides information about the risk involve in the consumption of milk in the Nyankpala community of Ghana. Also the antibiotic resistance patterns of

the *E. coli* as observed in this study provides a database by which other works can be compared. The Government of Ghana can use this data in coming up with policies regarding the use of antibiotics in the dairy industry.

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