ISSN 1819-1878

Asian Journal of Animal Sciences

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Asian Journal of Animal Sciences 9 (5): 233-240, 2015
ISSN 1819-1878 / DOI: 10.3923/ajas.2015.233.240

Antibiotic Resistance of *Escherichia coli* Isolated from Beef and its Related Samples in Techiman Municipality of Ghana

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ABSTRACT

In the present study, 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 45 *Escherichia coli* isolates were screened against nine different antibiotics. Overall, 34.57% of the *Escherichia coli* isolates were resistant, 7.16% were intermediate and 58.27% were susceptible. Resistance to vancomycin (88.89%) and erythromycin (68.89%) was high. Susceptibility to ciprofloxacin (95.56%), amoxycillin/clavulanic acid (86.67%), suphamethoxazole/ trimethoprim (82.22%) and gentamicin (75.56%) was also high. The *Escherichia coli* isolates also exhibited 25 antibiotic resistant patterns with the pattern VaE (vancomycin and erythromycin) and VaCCro (vancomycin, chloramphenicol and ceftriaxone) being the commonest (each exhibited by five different isolates). Multiple Antibiotic Resistance index (MAR index) ranged from 0.11-0.78. Resistance to seven (MAR index of 0.78) and five (MAR index of 0.56) different antibiotics was exhibited by 1 and 3 isolates, respectively. Some *Escherichia coli* isolates from different sources did exhibit the same resistance pattern. This study established the fact that *Escherichia coli* from meat and it related samples in Techiman Municipality were resistant to some antibiotics. Therefore, the use of antibiotics in the treatment of animals in the Municipality ought to be checked and controlled to prevent more isolates from becoming resistant. To the best of author's knowledge, this is the first report on the antibiotic resistance of *Escherichia coli* isolated from beef and its related samples in Techiman Municipality of Ghana.

Key words: Antibiotics, *Escherichia coli,* meat, susceptibility, resistance

INTRODUCTION

Escherichia coli are gram negative, facultative anaerobic and non-spore forming bacteria commonly found in the gastrointestinal tract of humans, farm animals, pests and wild animals (Anonymous, 2012; Malik *et al*., 2013; Kumar *et al*., 2013, 2014; Anita *et al*., 2014; CDC., 2014a). *Escherichia coli* cells are rod-shaped and are about 2.0 μm long and 0.25-1.0 μm in diameter, with a cell volume of 0.6-0.7 μm^3 (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; Anonymous, 2012). Eckburg *et al*. (2005) reported that *Escherichia coli* together with other facultative anaerobic constitute about 0.1% of gut flora. Beneficial *Escherichia coli* can produce vitamin K_2 for the host while pathogenic strains can cause bloody

diarrhoea, anaemia, urinary tract infection, meningitis, peritonitis and even death (Bentley and Meganathan, 1982; Von Baum *et al*., 2005). Faeco-oral transmission is one of the major routes through which pathogenic strains found their way into food or water and consequently cause disease (Schroeder *et al*., 2002; Aarestrup *et al*., 2008; Anita *et al*., 2014).

Escherichia coli have been isolated from beef, it related samples, water sources, humans, etc. have been implicated in foodborne outbreaks (Kilic *et al*., 2007; Adzitey *et al*., 2011, 2012a, 2013, 2014; Islam *et al*., 2011; Bekele *et al*., 2014; CDC., 2014b; Carnot *et al*., 2014). Adzitey *et al.* (2014) identified *Escherichia coli* in beef sold in the Yendi Municipality of Ghana. The prevalence of *Escherichia coli* O157:H7 in beef samples in Addis Ababa, Ethiopia was 13.3% (17/128) (Bekele *et al*., 2014). In USA, 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). 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 therapeutic purposes have been the major driving force behind the emergence and spread of drug-resistance bacteria among pathogenic and non-pathogenic bacteria strains (Schroeder *et al*., 2002; Aarestrup *et al*., 2008; Adzitey *et al*., 2012b). According to FDA (2014), surveillance data reveal that resistance in *Escherichia coli* is consistently the highest for antimicrobial agents that have long been in use in human and veterinary medicine.

Data on the antibiotic resistance of *Escherichia coli* in Ghana is scarce and in the Techiman Municipality of Ghana such data is unavailable. Therefore, this work report for the first time on antibiotic resistance of *Escherichia coli* isolated from beef and it related samples in Techiman Municipality of Ghana.

MATERIALS AND METHODS

Sources of *Escherichia coli* isolates: Forty five (45) *Escherichia coli* isolated from beef and its related samples between May 2013 and June 2014 in the Techiman Municipality of Ghana was used for this study. The *Escherichia coli* isolates were obtained from beef (n = 17), tables (n = 14) and knives (n = 14).

Antimicrobial susceptibility of *Escherichia coli*: The disk diffusion method of Bauer *et al*. (1966) was used to determine the antibiotic resistance of 45 *Escherichia coli* against the following antibiotics; amoxycillin/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, suphamethoxazole/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 resistant according to Clinical and Laboratory Standards Institute guidelines for (CLSI., 2006). 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.

RESULTS AND DISCUSSION

The antimicrobial susceptibility of 45 *Escherichia coli* were determined against 9 antimicrobial agents and the results are presented in Table 1. The overall resistance, intermediate and susceptibility was 34.57% (140/405), 7.16% (29/405) and 58.27% (236/405), respectively. A large percentage of the *Escherichia coli* were resistant to vancomycin (88.89%) and erythromycin (68.89%) but susceptible to ciprofloxacin (95.56%), amoxycillin/clavulanic acid (86.67%), suphamethoxazole/trimethoprim (82.22%) and gentamicin (75.56%). Intermediate resistances were observed for all the antibiotics except suphamethoxazole/trimethoprim, vancomycin and tetracycline. Intermediate resistance refers to those *Escherichia coli* species that were not clearly resistant or susceptible. 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*., 2012b). The use of antibiotics in the treatment of diseases and as growth promoters in farm animals, use of antibiotics for treating humans and other factors have been linked to the development of resistant microorganisms (Krumperman, 1983; Schroeder *et al*., 2002; Aarestrup *et al*., 2008; Adzitey *et al*., 2012b). Resistant *Escherichia coli* species can contaminate carcasses, processing equipment and other foods which pose a risk for public and animal health.

Table 2 shows the antibiotic resistance profile and Multiple Antibiotic Resistance (MAR) index of individual *Escherichia coli* from different sources. The *Escherichia coli* exhibited 25 antibiotic resistant patterns with MAR index ranging from 0.11-0.78. The majority of the *Escherichia coli* (14 isolates) were resistant to three antibiotics (MAR index of 0.33), followed by resistant to four antibiotics (13 isolates; MAR index of 0.44), resistant to two (10 isolates; MAR index of 0.22), resistant to one antibiotic (4 isolates; MAR index of 0.11), resistant to 5 antibiotics (3 isolates; MAR index of 0.56) and resistant to seven antibiotics (1 isolates; MAR index of 0.78). The resistance pattern VaE and VaCCro were the commonest and was exhibited by 10 (5 each) different *Escherichia coli* isolates. One *Escherichia coli* isolate was resistant to 7 different antibiotics and exhibited a resistant pattern of VaSxtAmcCTeCnE. Of the 3 *Escherichia coli* isolates that were resistant to five different antibiotics, 1 each exhibited the pattern VaSxtCTeE, VaCipTeCnE and VaAmcCCroE.

Studies on the antibiotic resistance of *Escherichia coli* isolates in Ghana are limited and the few studies available have also concentrated on human isolates. George *et al*. (2012) determined the antibiotic resistance patterns of *Escherichia coli* isolated from patients in selected hospitals in Kumasi, Ghana and reported that *Escherichia coli* isolates (28.6-46.4%) were resistant to gentamicin, ciprofloxacin and ceftriaxone while 14.4-47.4% gave intermediate responses. This study found 13.33% resistance to gentamicin, 2.22% resistance to ciprofloxacin and 26.67%

Table 1: Percentage antibiotic resistance of *Escherichia coli* isolated from meats and it related samples

		Escherichia coli			
Antimicrobial	Dose (µg)	*n/45	R (%)	I (%)	S (%)
Amc	30	2.00	4.44	8.89	86.67
C	30	20.00	44.44	2.22	53.33
Cip	5	1.00	2.22	2.22	95.56
Cro	30	12.00	26.67	11.11	62.22
Cn	10	6.00	13.33	11.11	75.56
E	15	31.00	68.89	28.89	2.22
Sxt	22	8.00	17.78	0.00	82.22
Te	30	20.00	44.44	0.00	55.56
Va	30	40.00	88.89	0.00	11.11

*n: No. of resistant *Escherichia coli*, S: Susceptible, I: Intermediate, R: resistant, Amc: Amoxycillin/clavulanic acid, C: Chlorofloxacin, Cip: Ciprofloxacin, Cro: Ceftriaxone, Cn: Gentamicin, E: Erythromycin, Sxt: Suphamethoxazole/trimethoprim, Te: Tetracycline, Va: Vancomycin

Table 2: Antibiotic resistance profile and multiple antibiotic resistance index of individual *Escherichia coli* from different sources

Escherichia coli code	Source	Antibiotic resistant profile*	No. of antibiotics	MAR index
TSM4	Meat	VaTeE	3	0.33
TMT1	Table	VaTeE	3	0.33
NAK3	Knife	VaTeE	3	0.33
SHT4	Table	VaTeE	3	0.33
AMT3	Table	VaTeCroE	4	0.44
SHM3	Meat	VaTeCnE	4	0.44
ATYK1	Knife	VaTeCnE	4	0.44
AYM4	Meat	VaTeCnE	4	0.44
ABM3	Meat	VaSxtTeE	4	0.44
TMM1	Meat	VaSxtTeE	4	0.44
SHT3	Table	VaSxtTeE	4	0.44
ZT2	Table	VaSxtTe	3	0.33
BGT3	Table	VaSxtCTeE	5	0.56
NAT3	Table	VaSxtCE	4	0.44
ABT2	Table	VaSxtAmcCTeCnE	7	0.78
ABK3	Knife	VaE	2	0.22
ATFK4	Knife	VaE	2	0.22
AFM1	Meat	VaE	2	0.22
NAM3	Meat	VaE	2	0.22
AYM3	Meat	VaE	2	0.22
SHK3	Knife	VaCTeE	4	0.44
STEM3	Meat	VaCTeE	4	0.44
ZM2	Meat	VaCTe	3	0.33
HSM3	Meat	VaCroE	3	0.33
BGM1	Meat	VaCroE	3	0.33
BGM3	Meat	VaCipTeCnE	5	0.56
AFK4	Knife	VaCE	3	0.33
HSM1	Meat	VaCCroE	4	0.44
HST3	Table	VaCCroE	4	0.44
SK1*	Knife	VaCCro	3	0.33
TSK1	Knife	VaCCro	3	0.33
DMK1	Knife	VaCCro	3	0.33
SHK1	Knife	VaCCro	3	0.33
STK4	Knife	VaCCro	3	0.33
DMM2	Meat	VaCCnE	4	0.44
STT4	Table	VaC	2	0.22
AYT1	Table	VaC	2	0.22
BGK4	Knife	VaAmcCCroE	5	0.56
ZM1	Meat	Va	1	0.11
STM1	Meat	Va	1	0.11
ST1	Table	TeE	2	0.22
STK2	Knife	SxtC	2	0.22
TMK2	Knife	E	1	0.11
TST1	Table	CTe	2	0.22
SK1	Table	Cro	1	0.11

Amc: *Amoxycillin/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, MAR: Multiple antibiotic resistance

resistance to ceftriaxone but intermediate responses were lower in this study (2.22-11.11%). In Accra, Ghana Namboodiri *et al.* (2011) reported that thirteen (52%) of 2006 and 2007 human *Escherichia coli* isolates and 10 (66.7%) of 2008 human *Escherichia coli* isolates were resistant to ciprofloxacin. The proportion of isolates resistant to each antimicrobial in 2008 was significantly greater than those seen in 2006 (Namboodiri *et al*., 2011). Anonymous (2015) analysed *Escherichia coli* isolated from hospitalized patients in Ghana for their sensitivity to antibiotics and reported that 82 and 75% were resistant to tetracycline and chloramphenicol, respectively. Resistance to tetracycline (44.44%) and chloramphenicol (44.44%) were lower in this study.

In other countries such as Canada, Carson *et al*. (2008) reported that *Escherichia coli* isolated from 29 volunteer beef farms in Ontario were all susceptible to ceftriaxone and ciprofloxacin. This work found some resistances to ceftriaxone and ciprofloxacin but susceptibility to ciprofloxacin was very high (95.56%). In USA, FDA. (2011) reported that *Escherichia coli* isolated from ground beef in 2011 were resistant to tetracycline (17.7%), trimethoprim-sulfamethoxazole (2.3%), chloramphenicol (1.4%), gentamicin (0.5%), amoxicillin-clavulanic acid (0.5%), ceftriaxone (0.5%) and ciprofloxacin (0.0%). Comparatively, this study found higher resistances to tetracycline, trimethoprim-sulfamethoxazole, chloramphenicol, gentamicin, amoxicillin-clavulanic acid, ceftriaxone and ciprofloxacin; even though resistance to amoxicillin-clavulanic acid (4.44%) and ciprofloxacin (2.22%) was low in this study (Table 1). In Bangladesh, *Escherichia coli* isolated from urinary tract infection patients were resistant to tetracycline (59%), ceftriaxone (5.5%), ciprofloxacin (11.5%) and gentamycin (8.1%) (Bhowmick and Rashid, 2004). Schroeder *et al*. (2002) also in the USA indicated that the prevalence of resistance among human *Escherichia coli* O157:H7 isolates was similar to that among cattle isolates for chloramphenicol (0 versus 1%), tetracycline (7 versus 11%) and amoxicillin-clavulanic acid (0 versus 1%). Furthermore, all *Escherichia coli* O157:H7 isolates, regardless of the source of isolation, were susceptible to ceftriaxone, gentamicin, ciprofloxacin and trimethoprim-sulfamethoxazole.

The antibiotic resistances reported in this study were either different from or similar to reports by other researchers in Ghana and other countries. These differences and similarities are due to differences in samples examined, differences in year/time of sampling, sampling procedure employed, breakpoints used and the level of usage of antibiotics in animal farming and human therapeutic purposes (Schroeder *et al*., 2002; Carson *et al*., 2008; Namboodiri *et al*., 2011; FDA., 2011). A wide variety of antibiotics have been suggested to be used in the treatment of infections caused by *Escherichia coli*. Pitout (2012) reported that the β-lactams, fluoroquinolones, aminoglycosides and trimethoprim-sulfamethoxazole are often used to treat community and hospital infections due to *E. coli*. β-lactams disrupt cell wall synthesis by binding to and inhibiting the penicillin-binding proteins essential for transpeptidation and carboxypeptidation reactions in cell wall peptidoglycan synthesis (Pitout, 2015). Fluoroquinolones interfere with DNA supercoiling and promote DNA gyrase-mediated double-stranded DNA. The aminoglycosides bind irreversibly to the 50S subunit of the 70S bacterial ribosomes (Pitout, 2015). Pitout (2015) also indicated that sulfonamides and trimethoprim interfere with bacterial folic acid synthesis by inhibiting tetrahydropteric acid syntheses and dihydrofolate reductase, respectively. Madappa (2014) showed that *Escherichia coli* meningitis, pneumonia and cholecystitis/cholangitis can be treated with third-generation cephalosporins such as ceftriaxone. *Escherichia coli* pneumonia can also be treated with fluoroquinolones and antimicrobials, known to be useful in cases of traveler's diarrhea include trimethoprim/sulfamethoxazole and fluoroquinolones (Madappa, 2014). In this study, most of the *Escherichia coli* isolates were susceptible to ciprofloxacin (95.56%), amoxycillin/clavulanic acid (86.67%), suphamethoxazole/trimethoprim (82.22%) and gentamicin (75.56%). Therefore, ciprofloxacin (fluoroquinolones) can be the first antibiotic of choice for treating *Escherichia coli* infection caused by the consumption of beef in the Techiman Municipality of Ghana. In the absence of ciprofloxacin, amoxycillin/clavulanic acid may be used before suphamethoxazole/trimethoprim and gentamicin.

CONCLUSION

In conclusion, *Escherichia coli* isolates from beef and its related samples exhibited varying resistances to antibiotics. Averagely, 58.27% were susceptible, 7.16% were intermediate and 34.57%

were resistant. High susceptibility was observed for ciprofloxacin, amoxycillin/clavulanic acid, suphamethoxazole/trimethoprim and gentamicin while high resistances were observed for vancomycin and erythromycin. Multiple antibiotic index ranged from 0.11-0.78 (that is resistant to 1-7 different antibiotics). Majority of the *Escherichia coli* isolates were resistant to three antibiotics, but the resistant pattern, VaE and VaCCro were the commonest. There is the need to control the use of antibiotics in animal farming and treatment of humans in Techiman Municipality of Ghana to control the incidence of increasing multidrug resistant *Escherichia coli* isolates. The findings of this study can serve as baseline information for the antibiotic resistance of *Escherichia coli* isolated from meat samples in Ghana to monitor trends in the future.

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