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Antibiotic susceptibility and production of extended-spectrum beta-lactamase (ESBL) of *E. coli* strains isolated from meat

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The zoonotic potential of *Escherichia coli* from chicken and beef food products is well documented. The uses of antibiotics on agriculture encourage the development of resistance bacteria capable of causing human disease and passing resistance to human pathogens. This study aimed to detect the antibiotic susceptibility and production of extended-spectrum beta-lactamase (ESBL) of *E. coli* strains isolated from meat. *E. coli* was isolated and identified according to standard techniques using traditional and chromogenic media and confirmed by biochemical reaction. Kirby-Bauer disk diffusion method was used to determine antimicrobial susceptibility towards twelve commonly used antibiotics. The resistance of the isolated *E. coli* towards the third generation of cephalosporins was detected using cefotaxime (30µg), ceftriaxone (30 µg) and ceftazidime (30 µg). ESBL producer *E. coli* was investigated using combination test. The results showed that 135 (75%) of the 180 meat samples revealed positive isolation of *E. coli*. 77.33% of the chicken meat samples showed positive isolation of *E. coli*, while 63.33% (19/30) of minced beef meat samples showed positive growth of *E. coli*. From these isolates, it was clear that most of them were highly resistant to tetracycline (10 µg), amoxiclav (30 µg) and cefalexin (30 µg). The lowest resistance was observed with ceftriaxone (30 µg) and ceftazidime (30 µg). The resistance of the isolated *E. coli* towards the third generation of cephalosporins was ranged between 5 to 33%. This study revealed that the isolated *E. coli* was ESBL producer as 85.71, 83.33, 70.83, 68.18 and 66.66% were detected in chickens leg, skin, wing, abdomen and chest respectively; while minced meat showed isolation of 15.78% of the ESBL producer *E. coli*. The study concluded that chicken and beef minced meat sold in Khartoum state have high hazardous risk for transmission of ESBLs producing *E. coli*; thus quality control application is highly needed. Policy actions should be implemented in order to prevent cross transmission of ESBLs producer *E. coli* to human.

Key words: *E. coli,* ESBL producer E. *coli*, susceptibility pattern, meat quality.

INTRODUCTION

Microbes in meat have a matter of great public health concern especially those causing food borne diseases (Pepin et al., 1997), particularly poultry, can be a source of ExPEC strain transmission to humans (Jakobsen et al.,

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2010; Overdevest et al., 2011; Manges and Johnson, 2012). Meats are especially common source of *Escherichia coli* contamination, which may be acquired during slaughter through fecal contact (Cohen et al., 2007).

Extraintestinal pathogenic *E. coli* (ExPEC) is an important group of pathogenic *E. coli* causing a diversity of infection in both animals and humans including septicemia, meningitis and urinary tract infections (UTIs). Also they are a major cause of economic loss to the poultry industry (Mellata, 2013; Köhler and Dobrindt, 2011; Russo and Johnson, 2003). Antibiotic resistance is a worldwide health problem in many fields, such as environment, livestock, human, veterinary medicine, and agriculture (Marshall and Levy, 2011). Livestock animals are considered important reservoirs of antibiotic- resistant Gram-negative bacteria (Aiello and Larson, 2003; Seiffert et al., 2013); these bacteria automatically bring antibiotic-resistant from animals to humans via consumption of meat. Major bacterial infections in humans can be traced back to livestock (Jakobsen et al., 2010; Overdevest et al., 2011). The overuse of antibiotics in food animal production contributes to increasing rates of antibiotic resistance (Ventola, 2015).

ESBL producing *E. coli* strains have emerged as a potential health hazard from food producing animals (Costa et al., 2009; Smet et al., 2008), they confer resistance to penicillins, cephalosporins and aminopenicillins including the third-generation cephalosporins cefovecin and ceftiofur and the fourth-generation cephalosporin cefquinome, which are approved veterinary drugs (Ewers et al., 2012; Madec et al., 2017). ESBL-producing *E. coli* has also been detected in wild animals, emphasizing the wide distribution of these resistance determinants (Guenther et al., 2011). Many studies have demonstrated the presence of ESBL-producing *E. coli* in animals and meat, most likely caused by the use of the third-generation cephalosporin ceftiofur in food animals. Since the late 1990s, ESBL-producing *E. coli* have been detected in retail meat and production animals in Europe, Asia, Africa, and the United States (Jouini et al., 2007; Blanc et al., 2006). This study aimed to detect presence of *E. coli* in frozen packed raw chicken meat and red minced meats, determine the antibiotic susceptibility of the isolated *E. coli* and to detect the presence of the extended spectrum beta lactamase enzyme in the isolated *E. coli*.

MATERIALS AND METHODS

Collection of samples

A total of 180 samples were collected as 150 samples of poultry included thirty samples from each part (wing, leg, abdomen, skin and chest). The chickens were purchased randomly from different factories in the Khartoum state from July 2015 to March 2018. The chicken samples were divided with sterile knives and assessors and kept separately in sterile collection bags at 4ºC. 30 minced beef meat from different company were purchased from super markets. Each package of minced meat was opened with sterile knife and forceps, and then collected in sterile bags.

Isolation and identification of *Escherichia coli*

Five grams of each food parts sample were blended by stomacher blinder. The samples were enriched in 45 ml Brain Heart Infusion Broth (Micromaster, Maharashtra-India) and incubated aerobically at 35°C for 3 h, then pre-enrichment by transferred to 45 ml of tryptone phosphate (TP) broth and incubated at 44ºC for 20 h. Each broth samples were inoculated on MacConkey agar medium (Himedia, Mumbai-India) and Eosin methylene blue (Levine) (Oxoid, Hampshire-England), then incubated aerobically for 18 h at 37°C; then confirmed by cultured on chromogenic agar (brilliance green *E. coli*/ coliform agar (Himedia, Mumbai-India) at 37ºC for 18 h. A positive *E. coli* bacteria were observed as purple colonies, then confirmed using indole test, vogues proskauer test, methyl red test, citrate test, motility test, Oxidase test and sugar fermentation test according to Cowan and Steel (1999).

Antibiotic susceptibility test

The standard Kirby-Bauer disk diffusion method was used to determine antimicrobial susceptibility of *E. coli* isolates according to Clinical and Laboratory Standards Institute guidelines (Clinical Laboratory Standards Institute Manual, 2013). The following antibiotics discs (Himedia, India) were tested: ciprofloxacin (5µg), cefixime (5µg), ceftazidime (30µg), ceftriaxone (30µg), cefotaxime (30 µg), amoxiclav (10µg), cefalexin (30µg), tetracycline (10µg), gentamicin (10µg), chloramphenicol (30µg), amikacin (30µg), and co-trimexazole (30µg). 18 h broth cultures were prepared and equivalent to the 0.5 McFarland turbidity standards. The antibiotic discs were impressed on inoculated plates and incubated at 37^{o}C for 24 h. Diameter of inhibition zones of *E. coli* isolates around each antimicrobial disc was measured in mm, then the results were reported as sensitive (S), intermediate (I), and resistant (R).

Screening of extended spectrum beta lactamase enzyme production

The discs of antibiotics containing cephalosporin alone (cefotaxime 30 ug, ceftazidime 30 ug, ceftriaxone 30 ug) and in combination with clavulanic acid were applied onto isolated *E. coli* inoculated plates, and then sufficient space between individual discs was ensured to allow proper measurement of inhibition zones. The plates were incubated at 37°C for 18 h. The inhibition zone around the cephalosporin discs combined with clavulanic acid was compared with the zone around the disc with the cephalosporin alone. The test is positive (ESBL producer) if the inhibition zone diameter is ≥ 5 mm larger with combined cephalosporin and clavulanic acid than cephalosporin alone.

RESULTS

Number and percentage of the isolation of *E. coli*

E. coli was identified according to the morphological culture characteristic and biochemical reactions as shown in Table 1 and Figure 1. *E. coli* was isolated from 135 (75.00%) out of 180 samples, while the negative isolation

Table 1. Identification of *E. coli.*

Test	Isolated *E. coli* reaction	Test	Isolated *E. coli* reaction
Gram stain	Gram negative	MR	Positive
Motility	Motile	VP	Negative
Oxidase production	Negative	Citrate	Negative
Catalase production	Positive	Growth on MacConkey agar	Pink colonies
Indole production	Positive	Growth on chromogenic media	Purple colonies

Figure 1. Purple colonies of *E.coli* chromogenic agar (brilliance green *E. coli*/ coliform agar).

Table 2. Number and percentage of *Escherichia coli* isolated from meats.

Samples	Number of samples	No. (%) of positive isolation of *E . coli*	No. (%) of negative isolation of *E. coli*
Chicken Inside wing	30	24 (80.00)	6(20.00)
Chicken Chest	30	18 (60.00)	12(40)
Chicken Abdomen	30	22 (73.33)	8(26.66)
Chicken Leg	30	28 (93.33)	2(6.66)
Chicken Skin	30	24 (80.00)	6(20.00)
Red minced meat	30	19 (63.33)	11(36.66)
Total number	**180**	**135 (75.00)**	**45 (25%)**

of *E. coli* was observed in 45 (25%). The highest number of isolation of *Escherichia coli* was recorded generally in chicken meats part samples as 116 (64.44%), while red minced meats showed percentage of 63.33%. There were variations in the number of isolation of *E coli* among chicken meats. The highest isolates was observed in leg part samples as 28 (93.33%) out of 30 samples, followed by both inside wing and skin part which showed isolation of 24 (80.00%) out of 30 samples of each. The abdomen revealed 22 (73.33%) out of 30 samples, while chest showed 18 (60.00%) out of 30 samples. A positive isolates of *E. coli* from red minced meat were 19 (63.33%) out of 30 samples (Table 2).

Antimicrobial susceptibility of the isolated *E. coli*

The antibiotic resistance pattern of the isolated *E. coli* from wing parts of frozen chicken meats was recorded as 79.2% resistance against tetracycline, 66.7% towards both of gentamicin and amoxiclav, while cefalexin and co-trimexazole were resistant with percentage of 45.8%. *E. coli* isolated from skin of the chicken showed highest percentage of resistance towards tetracycline and amoxiclav as 70.8% and Cefalexin as 54.2%. On the other hand, all isolated *E. coli* showed clear sensitivity towards ceftriaxone. Similarly *E. coli* isolated from chest part of the chickens showed the highest percentage of resistance for tetracycline (72.2%) followed by amoxiclav (55.6%), cefalexin (50%) while all isolates (100%) showed clear sensitivity towards both ceftriaxone and ceftazidime. The *E. coli* isolated from the abdomen of the chicken showed resistance towards cephalexin and tetracycline as 63.6% and cefixime as 54.5%. The isolated *E. coli* showed resistance to amoxiclav and gentamicin with percentage of 50% (Table 3). On the

Table 3. Percentage of the resistance of the isolated *E. coli* towards antibiotics.

Antibiotics (µg)	Percentage of the resistance of isolated *E. coli*					
	Chicken					Cattle red minced Meat
	Wing	Skin	Leg	Chest	Abdomen	
Cefalexin (30)	45.8	54.2	50	50	63.6	31.6
Ceftriaxone (30)	4.2	0	7.1	0	4.5	10.5
Cotrimexazole (25)	45.8	29.2	35.7	38.9	36.4	15.8
Gentamicin (10)	25	45.8	46.4	16.3	50	10.5
Cloramphenicol (30)	41.7	37.5	39.3	38.9	36.4	10.5
Cetazidime (30)	8.3	8.3	3.6	0	4.5	5.3
Tetracycline (10)	79.2	70.8	57.1	72.2	63.6	26.3
Amikacin (30)	16.7	41.7	39.3	22.2	18.2	15.8
Ciprofloxacin (5)	29	29.2	32.1	22.2	31.8	0
Cefixime (5)	37.5	50	32.1	44.4	54.5	57.9
Cefotaxime (30)	33.3	12.5	17.9	22.2	18.2	5.3
Amoxiclav (30)	66.7	70.8	17.9	55.6	50	31.6

Table 4. Percentage of resistance of isolated *E. coli* towards third generation of cephalosporins**.**

Antibiotic (30 µg)	Percentage of resistance of isolated *E. coli*					
	Chest	Abdomen	Skin	Leg	Wing	Cattle red minced meat
Ceftriaxone	0	5	0	7	4	11
Ceftazidime	0	5	8	4	8	5
Cefotaxime	22	18	13	18	33	5

other hand *E. coli* isolated from minced meat showed lower resistance towards tested antibiotics. The highest resistance was observed with cefixime as 57.9%, followed by cefalexin and amoxiclav as 31.6% for both. All isolated *E. coli* from minced meat were sensitive to ciprofloxacin.

Resistance of the isolated *E. coli* towards third generation of cephalosporins

The study revealed that the highest percentage of resistance to third generation cephalosporins were detected in chicken parts, especially wing parts as 33% to cefotaxime followed by ceftazidime (8%) and ceftriaxone (4%) compared with *E. coli* isolated from red minced meats which showed 11% resistance for ceftriaxone, 5% to both cefotaxime and ceftazidime. The highest percentages of resistance were detected in *E. coli* isolated from chicken parts towards cefotaxime which were distributed as follows: wing parts, 33%, chest parts, 22%; both abdomen and leg 18%; and skin 13%. All isolated *E. coli* from chest parts showed clear sensitivity towards ceftriaxone and ceftazidime. In skin parts isolates, no resistance was observed towards ceftriaxone. Among all isolates of *E. coli* from red minced meats, the highest rate of resistance towards ceftriaxone was 11% (Table 4).

Detection of extended spectrum beta lactamase enzyme

The results revealed that out of 116 isolates of *E. coli* from chicken samples, 75.86% were positive as ESBL *E. coli* producer, while out of 19 isolates of *E. coli* from red minced meat samples only three of them showed production of ESBL enzyme (15.78%). Among the isolated *E. coli* from chicken meats parts, the leg part represented the highest percentage of isolation of ESBL *E. coli* producer (24%), followed by skin samples (20%), wing samples (17%); whereas abdomen samples represented 15% (Table 5).

DISCUSSION

In this study, 75% of the collected samples revealed positive growth of *E. coli.* The isolation of *E. coli* indicates low quality of food, fecal contamination and the presence

Table 5. Percentage of ESBL producer *E. coli* from chicken meats and cattle red minced meat.

Test	Percentage of positive isolation of ESBL *E. coli*					
	Cattle red minced meat	skin	abdomen	wing	leg	chest
Positive for combination test	3(15.78%)	20(83.33%)	15(68.18%)	17(70.83%)	24(85.71%)	12(66.66%)
Number of the positive *E. coli*	19	24	22	24	28	18

of high risk of transmission of enteric dangerous pathogens; otherwise some strain of *E. coli* is considered as pathogens that causes very serious disease. In fact, during and after slaughtering, the bacteria from the animal microbiota, the slaughter house environment, hands and equipment might contaminate carcasses. Some of these bacteria may grow and survive during storage, other pathogenic bacteria such as *Salmonella, Listeria, Campylobacter*, *Aeromonas, Staphylococcus* and toxin producing aerobic and anaerobic gram positive bacteria might be present. These confirm the findings of Authority (2016), Praveen et al. (2016), Höll et al. (2016), Line et al. (2013), and Veluz et al. (2012) who reported isolation of *E. coli* and pathogenic bacteria from poultry meat.

In this study, the isolated *E. coli* showed high resistance towards many commonly used antibiotics, these strongly pointed to the misuse of the antibiotic in animal production sector. Antimicrobials are used extensively in food animal production for disease prevention, treatment and growth promotion. Sarma et al. (1981) discovered that approximately 80% of isolated *E. coli* from healthy and diseased poultry was resistant to chlortetracycline, tetracycline, oxytetracycline and triple sulfas. Similarly, Paula Signolfi et al. (2019) found that more than 67% of isolated *Escherichia coli* were resistant to tetracycline, nalidixic acid and ampicillin. The inappropriate use of antibiotics, not only in human medicine but also in animal husbandry has been considered a main driver leading to the increase of multi-drug resistant bacteria (Chantiziaras et al., 2014). The higher rates of antimicrobial resistance and multi-drugs resistance of the isolated *E. coli* in this study could be due to poor monitoring by regulatory bodies as the use of antibiotics in animal farms that used production of meat for human consumption, which have been prohibited in several countries. Furthermore, transmission via consumption of meat products has been suggested as a potential source of multidrug resistant bacteria in Africa (Alonoso et al., 2017; Eibach et al., 2018).

The increasing incidence of infections caused by extended-spectrum beta-lactamase (ESBL) *Escherichia coli* is of serious concern, as many studies from countries with a highly industrialized poultry industry suggested that meat products of poultry farms might be an important source for transmission of (ESBL) *Escherichia coli* to human (Linda et al., 2019; Poirel et al., 2018). Hawkey (2008), Kumarasamy et al. (2010) and Mathai et al. (2002) found that 70-90% of *Enteroacteriaceae* are ESBL producers in India. Kar et al. (2015) conducted systematic study on multiple drugs resistant ESBL producing *E. coli* in food producing animals. Paula Signolfi et al. (2019) found that more than 31% of the isolated *Escherichia coli* were ESBl producer. Furthermore, ESBL producer *E.coli* was found to be more resistant to a higher number of antimicrobial substances compared to non ESBL producing *E.coli.* Alonso et al. (2017) reviewed lower prevalence of (ESBL) *Escherichia coli* among poultry meat products in African countries compared with European countries. However previous studies did not use any (ESBL) screening plates for the detection of *Escherichia coli* which might under estimate the ESBL production. Studies from the Netherland, Sweden and Vietnam detected (ESBL) *Escherichia coli* not only in chickens but also in high numbers among humans (Borjesson et al., 2016). These studies concluded that poultry farms or meat products might be an important source of (ESBL) *Escherichia coli.* Furthermore, ESBL strains of *Escherichia coli.* were found to be 1.40 times more likely to contain more virulence genes than non ESBL – producing strain and it could be transmitted to human via food chain.

The percentage of the isolation of third generation cephalosporin resistant *E. coli* varied in this study according to meat source and parts in chicken, wing, chest, abdomen, legs and skin showed resistance of 33, 22, 18, 18 and 13% respectively towards cefotaxime (30 µg). These results indicate the highest use of cefotaxime in poultry farms and confirm the fact that the broiler farms are beginning to shift to more recently developed drugs, such as third generation cephalosporin as mentioned by Zekar et al. (2017). Third generation cephalosporin are used to treat urinary tract infections caused by Gram negative bacteria and have recently received research attention because of the rapid spread of multidrug-resistance. This resistance related to a novel gene called *fos* A3, which has been reported in *Escherichia coli* and *Klebsiella pneumonia* and often detected in bla $_{CTX-M}$ producing and multi-resistant *Escherichia coli* both in animals and in clinical isolates (Ho et al., 2013). These findings raised the possibility that *Escherichia coli* present in the intestinal tract of healthy individuals could acquire those genes from *Escherichia coli* derived from chicken meat, which could act as reservoir for bacteria harboring resistance genes (Manges and Johnson, 2012). This study concluded that there is a high need for application

of the quality control measurements to ensure serving good and safe food as well as prevent transmission of food borne pathogen and control the rise of antimicrobial resistance microorganisms.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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