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Characterization of *Escherichia coli* isolates obtained from washing and rinsed water of broilers in pluck shops at Sreepur of Gazipur district in Bangladesh

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ABSTRACT

The study was aimed at characterization of *Escherichia coli* isolates obtained from washing and rinsed water of broilers in pluck shops at Sreepur of Gazipur district in Bangladesh. A total of 30 samples collected from the different layers of drums of pluck shops' were subjected to bacterial isolation and identification by using cultural and biochemical techniques. Furthermore, the isolated *E. coli* strains were characterized by antimicrobial susceptibility testing. The fermentation reaction by the isolates of *E. coli* in five basic sugars (dextrose, sucrose, fructose, maltose, mannitol) and in dulcitol were positive. Moreover, methyl red reaction and catalase tests were also positive for *E. coli*. On the other hand, *E. coli* was prevailed in upper layer of drums (31.82%), in middle layer of drums (36.36%) and in lower layer of drums (31.82%). *E. coli* isolates were resistant to erythromycin and enrofloxacin. However, most of the *E. coli* isolates were susceptible to sulfamexazole-trimethoprim and gentamycin. Out of 22 *E. coli* isolates, 16 (72.73%) were multidrug resistant. The findings of the study revealed the presence of multidrug resistant *E. coli* isolates in washing and rinsed water of broilers in Pluck shops at Sreepur of Gazipur district in Bangladesh.

1. Introduction

About 89% of the rural households' in Bangladesh rear poultry (Bangladesh Bureau of Statistics, 1996). Such rural farming is emerging as a strong agro-based industry including the backyard poultry rearing system to commercial intensive one, during the last two decades in Bangladesh. Undoubtedly the poultry slaughtered, eviscerated and dressed under local conditions carry contaminants loading from the point of slaughtering process to the point at which the consumers are offered. There occurs biomagnifications at all levels of handling, poor transport and retailing conditions. The contaminants may be fecal, offals, carriage vehicles, cages and also from the feeds and wastes of poultry. As contaminants *Escherichia coli* is the vital bacterial organism during the slaughtering, handling, packaging and washing process. Use of antimicrobials in any environment creates selection pressures that favor the survival of antibiotic-resistant pathogens. The routine practice of giving antimicrobials to domestic livestock for growth promotion and prophylaxis is an important factor in the emergence of antibiotic-resistant bacteria in the food chain (Tollefson et al., 1997). At slaughter, resistant strains from the gut readily soil poultry carcasses and as a result poultry meats are often contaminated with multiresistant *E. coli* (Caudry et al., 1979; Nazer, 1980; Bensink and Botham, 1983; Linton et al., 1977; Turtura et al., 1990). Hence, resistant faecal *E. coli* from poultry can infect humans both directly and via food. Information concerns to bacterial loads during slaughtering, washing, handling and packaging by the retail shopkeepers, renders or the consumers are not available to get the safe consumption of poultry meat. Therefore, the present study was undertaken to isolate, identify and characterize *Escherichia coli* from washing and rinsed water of broilers in pluck shops.

2. Materials and methods

2.1. Study Area

The present research was conducted during the period of December 2011 to May 2012 in the Bacteriology laboratory of the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh. The samples (Broiler washing and rinsed water) which were collected from Pluck shops (cottage poultry processors) at Gorgoria-masterbari, Sreepur of Gazipur district (Figure 1) and transported through ice flasks to the laboratory of the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh for isolation, identification, biochemical and antibiogram characterization.

2.2. Collection and transportation of samples

A total of 30 samples were collected from source water and 3 layers of washing and rinsed water of drums of pluck shops' at Gorgoria-masterbari, Sreepur, Gazipur and immediately brought to Bacteriology Laboratory of the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh through cool chain maintaining. After that, the samples were inoculated into the Nutrient broth for better nourishment of the desirable organisms.

2.3. Biochemical studies for the identification of *E. coli* isolates

Several biochemical tests were performed for the confirmation of the isolates as *E. coli.* The biochemical tests performed were sugar fermentation tests, indole test, methyl red (MR) test, Voge's Proskauer (VP) test and Citrate test as described by Cheesbrough, 2000. *E. coli* were characterized by their ability to ferment glucose, lactose (for some strains it is negative), maltose and produce gas (CO_2), positive for indole test and MR test and negative for VP and Citrate utilization test.

2.4. Motility Test of *E. coli* Isolates

The motility test was performed according to the method described by Cowan (1985) to differentiate motile bacteria from the non-motile one. Before performing the test, a pure culture of the *E. coli* isolates was allowed to grow in nutrient broth. One drop of cultured broth was placed on the cover-slip and placed inverted over the concave depression of the hanging drop slide to make hanging drop preparation. Vaseline was used around the concave depression of the hanging drop slide for better attachment of the cover-slip to prevent air flow and evaporation of the fluid. The hanging drop slide was then examined carefully under 100x objective of a compound microscope using immersion oil. The motile and non-motile organisms were differentiated by observing motility in

contrasting with to and fro movement of bacteria. *E. coli* show the same characteristics as *Salmonella* in MIU medium (a spreading turbidity from the stab line or a turbidity throughout the medium).

2.5. Antimicrobial Susceptibility Test

Susceptibility and resistance of different antibiotics was measured in vitro by employing the modified Kirby-Bauer (Quinn et al., 2000) method. This method allowed for the rapid determination of the efficacy of a drug by measuring the diameter of the zone of inhibition that resulted from diffusion of the agent into the medium surrounding the disc.

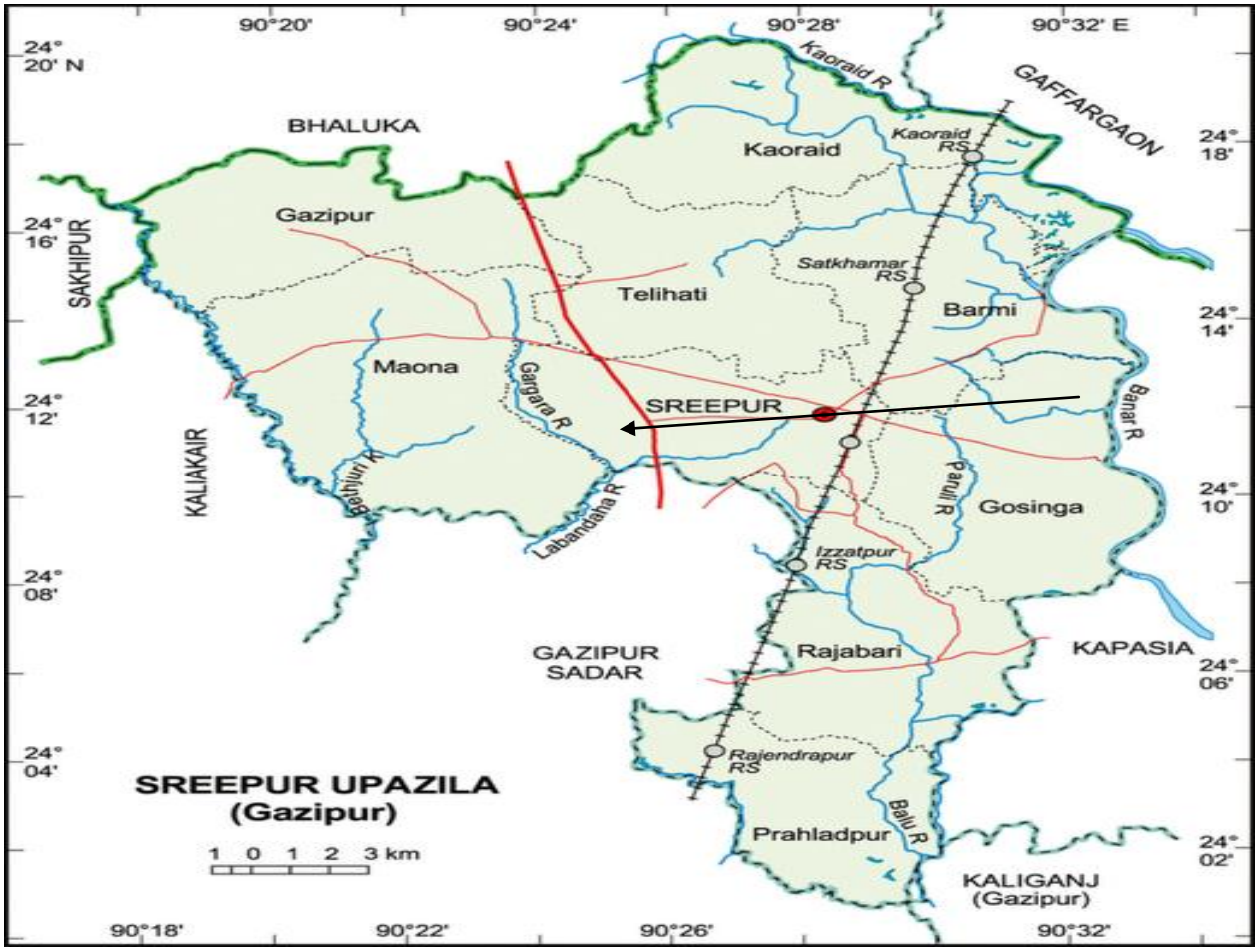

Fig. 1. Map of Sreepur Upazila of Gazipur District (Source: Banglapedia).

A suspension of test organism was prepared in nutrient broth by overnight culture for 24 hours at 37 ºC. The broth were streaked using by sterile glass spreader homogenously on the medium. Antibiotic disc were applied aseptically to the surface of the inoculated plates at an appropriate special arrangement with the help of a sterile forceps on Mueller-Hinton agar plates. The plates were then inverted and incubated at 37 ^{0}C for 24 hours. The diffusion discs with antimicrobial drugs were placed on the plates and incubated for 24 hours at 37 ºC. The antibiotics discs (Oxoid, Basingstoke, Hampshire, England) used were: amoxycillin (30 µg), neomycin (30 µg), amikacin (30 µg), erythromycin (15 µg), gentamicin (10 µg), enrofloxacin (5 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), doxycycline (30 µg), erythromycin (10 µg), and sulphamethoxazole-trimethoprim (25 µg). Sterile glass spreader was used to spread the culture homogenously on the medium. Antibiotic disc were applied aseptically to the surface of the inoculated plates at an appropriate special arrangement with the help of a sterile forceps. The plates were then inverted and incubated at 37 ^{0}C for 24 hours. After incubation, the plates were examined and the

diameters of the zone of complete inhibition were observed. The zone diameters for individual antimicrobial agents were translated into susceptible, intermediate and resistant categories by referring to an interpretation table (Table 1).

Table 1
Interpretive standards for disk diffusion susceptibility testing of Enterobacteriaceae

Name of Antimicrobial Disk	Disk concentration	Diameter of zone of Inhibition (mm)		
		Susceptible	Intermediate	Resistant
Neomycin (N)	30 µg	≥ 17	13~16	≤12
Amikacin (AK)	30 µg	≥ 17	15~16	≤ 14
Erythromycin (E)	15 µg	≥ 23	14~22	≤13
Gentamicin (CN)	10 µg	≥ 15	13~14	≤12
Ciprofloxacin (CIP)	5 µg	≥ 21	16~20	≤15
Doxycyclin (DO)	30 µg	≥ 16	13~15	≤12
Norfloxacin (NOR)	10 µg	≥ 17	13~16	≤12
Amoxycillin (AMC)	30 µg	≥ 18	14~17	≤13
Enrofloxacin (ENR)	5 µg	≥ 20	17~19	≤16
Sulfamethoxazole-trimethoprim(SxT)	25 µg	≥ 16	11~15	≤10

(NCCLS, 2000 and Bauer et al., 1966).

2.6. Maintenance of stock culture

During the experiment it was necessary to preserve the isolated *E. coli* organisms for longer period. Preservation of *E. coli* isolates in pure culture form was stored in sterile 80% glycerin and was used as stock culture. The equal volume of 80% glycerin and bacterial culture were mixed and capped tightly and stored at -70 ^{0}C. The isolated organisms were given code name for convenience.

3. Results

A total of 22 bacterial isolates out 30 samples were identified as *E. coli* by using cultural and biochemical techniques. The results of cultural, morphological and motility characteristics of the isolates of *Escherichia coli* are presented in Table 2.

Among all of the isolates obtained from this study *E. coli* produced turbidity from line of inoculation in Motility Indole Urea media (MIU) which is a sign of motility and alternatively found to be motile with hanging drop slide (Table 2).

The organisms were checked and confirmed by their purity using selected media (SS, EMB agar). Then they were grown in nutrient broth for biochemical test. For identification, a series of selective biochemical tests for *E. coli* were performed with the culture (Table 3). All the isolated organisms fermented five sugars like Dextrose, Sucrose, Fructose, Glucose, Maltose produced acid with gas. Acid production was indicated by the colour change from reddish to orange yellow color and the gas production was manifested by the appearance of gas bubbles in the inverted Durham's tubes (Table 3). All the isolates of *E. coli* were found positive in catalase, Methyl-red test, Indole test, and VP test negative (Table 3).

E. coli was prevailed as 31.82% in upper layer of drums, 36.36% in middle layer of drums and 31.82% in lower layer of drums (Table 4).

The results of the antimicrobial susceptibility testing by disc diffusion method with 10 chosen antimicrobial agents are presented in (Table 5). Out of 22 *E. coli* isolates, 22 (100%) were resistant to Erythromycin, 14 (63.64%) were resistant to Enrofloxacin, 11 (50%) were resistant to Doxycyclin. Further more, 14 (63.64%) and 12 (54.55%) were intermediate resistant to Neomycin Ciprofloxacin respectively. On the other hand, 22 (100%) were susceptible to Sulfamethoxazole-Trimethoprim. The results of antimicrobial resistance patterns of *E. coli* isolates are summarized in (Table 6). Out of 22 *E. coli* isolates, 2 (9.09%) and 1 (4.55%) were resistant to each of 6 antibiotics; in where 4 (18.18%), 1 (4.55%) and 1 (4.55%) were respectively resistant to each of 2 antibiotics. Among 22 *E. coli* isolates, 16 (72.73%) were multidrug resistant when considered resistant to 2 or more antimicrobial agents.

Table 2
Results of cultural, morphological and motility characteristics of the isolates of *Escherichia coli.*

Sources of isolates	Colony morphology					Staining characteristics	Motility (Both in MIU medium & Hanging drop method)
	Nutrient agar	SS agar	BG agar	EMB agar	MC agar		
S1 to S30 (Except :S-2,5,7,14,20,25,28 ,29)	circular, raised, smooth, colorless colony	pinkish, circular, small colony	greenish yellowish colonies	Black metallic sheen colonies	bright pink colonies	Pink short rod, gram negative bacilli	+ve

N.B: SS = Salmonella-Shigella; BG = Brilliant Green; EMB = Eosine-Methylene Blue ,
MC = MacConkey; + = Positive, S=Sample (1……..30). MIU=Motility Indole Urea.

Table 3
Different biochemical test of Escherichia coli

Fermentation reaction with five sugars(1)	Result	Other biochemical reaction	Result
1. a. Dextrose	+	2. Indole	+
1. b. Sucrose	+	3. MR	+
1.c. Fructose	+	4. VP	-
1.d. Maltose	+	5. Dulcitol test	+
1.e. Mannitol	+	6.Catalase	+

N.B: + = Positive; - = Negative; MR = Methyl red; VP = Voges Proskauer

Table 4
Frequency percentage of the isolated *E. coli* (n = 22) in source samples.

Source of samples	Percentages (%)
Upper layer of drums	31.82
Middle layer of drums	36.36
Lower layer of drums	31.82

Table 5
Results of Antimicrobial susceptibility of *E. coli.*

Name of isolates	No. (%)									
E. coli (n=22)	**N**	**AK**	**E**	**AMC**	**ENR**	**CN**	**DO**	**CIP**	**NOR**	**SxT**
S	6(27.27)	20(90.91)	0(0)	11(50)	0(0)	18(81.82)	5(22.73)	7(31.82)	19(86.36)	22(100)
I	14(63.64)	1(4.55)	0(0)	5(22.73)	8(36.36)	2(9.09)	6(27.27)	12(54.55)	3(13.64)	0(0)
R	2(9.09)	1(4.55)	22(100)	6(27.27)	14(63.64)	2(9.09)	11(50)	3(13.64)	0(0)	0(0)

Neomycin (N), Amikacin (AK), Erythromycin (E), Gentamicin (CN), Ciprofloxacin (CIP), Doxycyclin (DO), Norfloxacin (NOR), Amoxycillin (AMC), Enrofloxacin (ENR), Sulfamethoxazole-Trimethoprim(SxT).

4. Discussion

This study was aimed at isolation, identification and biochemical differentiation of Escherichia coli from the washing and rinsed water collected from Pluck shops (cottage poultry processors) at Gorgoria-masterbari, Sreepur, Gazipur district and Antibiogram characterization of desirable microorganisms were also accomplished. Most of source water of pluck shopkeepers' was direct Tube-well water while the two sources were stored tanks' water from local water pump. Only of 30 sample source, we detected *E. coli* in 22 samples.

Isolation and identification results of the study also indicated that the field sample contained Gram negative, rod shape and motile organism with various colony characteristics (large, smooth, round and sticky) in different bacteriological media. The isolate was able to produce characteristic black metallic sheen colonies on EMB agar, pink colony on, pinkish colony on SS agar, circular, raised, smooth, colorless colony on nutrient agar and greenish yellowish colonies in BGA as well. The colony characteristics of the isolated *E. coli* in different media resemble the colony characteristics of *E. coli* as stated by Escherich (1885) and Ali et al. (1998).

Table 6
Results of Antimicrobial resistance pattern of *E. coli* isolates.

Isolates	Resistance profiles	No. of isolates (%)
	No resistance demonstrated	-
E. coli (n=22)	Resistant to 1 agent (E)	6 (27.27)
	a. Resistant to 2 agents (E- ENR)	4 (18.18)
	b. Resistant to 2 agents (N-E)	1 (4.55)
	c. Resistant to 2 agents (E-DO)	1 (4.55)
	Resistant to 3 agents (E- ENR-DO)	4 (18.18)
	Resistant to 4 agents (E- ENR-AMC -DO)	2 (9.09
	Resistant to 5 agents (E- ENR-AMC –DO-CIP)	1 (4.55)
	a. Resistant to 6 agents (E- ENR-AMC –DO-CN-CIP)	2 (9.09)
	b. Resistant to 6 agents (N-AK-E- ENR-AMC –DO)	1 (4.55)
	Resistant isolates	n=22 (100%)

Neomycin (N), Amikacin (AK), Erythromycin (E), Gentamicin (CN), Ciprofloxacin (CIP), Doxycyclin (DO), Norfloxacin (NOR), Amoxycillin (AMC), Enrofloxacin (ENR),Sulfamethoxazole-Trimethoprim(SxT).

The fermentation reaction by the isolates of E. coli in five basic sugars (dextrose, sucrose, fructose, maltose and mannitol) and in dulcitol was positive. More over, MR reaction and Catalase tests were also positive for *E. coli*. The organism was able to ferment lactose, dextrose and mannitol, sucrose and maltoses completely. The result of sugar fermentation agreed with the findings of Beutin et al. (1997) and Sandhu et al. (1996). These respective authors reported that although *E. coli* ferments all five basic sugars but it partially fermented sucrose and maltose. Variation of the results might be due to genetic factors and nature of inhabitant of the organisms.

Antimicrobial resistance testing was performed by disc diffusion method using 10 different antibiotics. In antimicrobial susceptibility testing, out of 22 *E. coli* isolates, 22 (100%) were resistant to erythromycin, 14 (63.64%) were resistant to enrofloxacin, 11 (50%) were resistant to doxycyclin. Further more, 14 (63.64%) and 12 (54.55%) were intermediate resistant to neomycin, ciprofloxacin respectively. On the contrary, 22 (100%) were susceptible to sulfamethoxazole-trimethoprim though which were very close to the findings of Hossain (2006). A total of 22 *E .coli* isolates, 16 (72.73%) were multidrug resistant, these findings are in partial agreement with Kabir, 2010; Nazir et al. 2005; Rahman et al. 2004; Islam et al. 2004.

E. coli were isolated and characterized successfully from broiler washing and rinsed water of broilers in pluck shops at Sreepur of Gazipur district in Bangladesh using different cultural, morphological examination, biochemical and antimicrobial susceptibility test. The findings of the present study revealed the presence of multidrug resistant *E. coli* isolates in washing and rinsed water of broilers in Pluck shops. Further molecular studies on the isolated *E. coli* strains will be required for better understanding of their clonality and mechanisms of antimicrobial resistance.

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