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Antibiotic Resistance of *Escherichia Coli* Isolated From Poultry and Poultry Environment of Bangladesh

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Abstract: Problem statement: Increased emergence in microbial resistance to antibiotics is a growing problem in Bangladesh, a tropical country with a large agrarian population having limited medical facilities. Wide spread use of antimicrobials in poultry farming here is a concern of multi-drug microbial resistance development that can potentially be transmitted to human pathogens even from non-pathogenic carrier strains. Attempt was made to assess drug susceptibility in *Escherichia coli* from poultry sources of Bangladesh. **Approach:** Eighty selected strains isolated from poultry sources were thoroughly characterized by standard cultural and biochemical tests followed by final identification using latex agglutination test of polyvalent anti-sera, from which 50 were tested for susceptibility to 13 antibiotics following disk diffusion method. **Results:** 145 (58 %) samples, out of total 250, were found positive for *E. coli*. 52-88 % of tested *E. coli* strains from poultry sources were found resistant to Penicillin, Ciprofloxacin, Riphampicin, Kanamycin, Streptomycin, Cefixine, Erythromycin, Ampicillin, Tetracycline, and 20 % strains showed resistance to both Chloramphenicol and Neomycin. No strains showed resistance to Norfloxacin and Gentamicin. Sensitivity was recorded in case of 60-86 % strains to Norfloxacin, Gentamicin, Chloramphenicol, and Neomycin; and 26-36 % strains against Tetracycline, Streptomycin, and Ampicillin. Intermediate resistance/ susceptibility to various antibiotics were observed for 12-36 % *Escherichia coli* strains. Both, resistance and susceptibility were exhibited against Chloramphenicol, Ampicillin, Gentamicin, Neomycin, Tetracycline, Streptomycin and Norfloxacin. Multi drug resistance was found in case of 6-10 antibiotics for all strains tested. **Conclusion:** Further study is required on the role of poultry borne bacteria as vectors in transmitting drug resistance. Attention is to be paid for personnel hygiene in processing and handling of poultry and poultry products; and excess use or abuse of antibiotics should be reduced or stopped by the judicious application of antibiotics for the safety of public health in Bangladesh.

Key words: *Escherichia coli*, antibiotic resistance, poultry environment, Bangladesh

INTRODUCTION

Escherichia coli is one of the common microbial flora of gastrointestinal tract of poultry and human being including other animals but may become pathogenic to both[1,2]. Although most isolates of *E. coli* are nonpathogenic but they are considered as indicator of fecal contamination in food and about 10-15 % of intestinal coliforms are opportunistic and pathogenic serotypes[3] and cause a variety of lesions in immunocompromised hosts as well as in poultry. Among the diseases some are often severe and sometimes lethal infections such as meningitis, endocarditis, urinary tract infection, septicemia, epidemic diarrhea of adults and children[4] and yolk sac infection, omphalitis, cellulitis, swollen head syndrome, coligranuloma, and colibacillosis[5]. During the past two decades, severe outbreaks of gastrointestinal illness have occurred by food borne pathogenic *E. coli*, especially 0157:H7[6].

Antibiotics are extensively used as growth promoters in poultry production or to control infectious disease. Anti-microbial exercise and/or especially abuse is considered to be the most vital selecting force to antimicrobial resistance of bacteria[7,8]. Moreover, antibiotic treatment is considered the most important issue that promotes the emergence, selection and spreading of antibiotic-resistant microorganisms in both

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veterinary and human medicine[9,10]. It was stated by well established evidence that antibiotics can lead to the emergence and dissemination of resistant *E. coli* which can then be passed into people via food or direct contact with infected animals. These resistant microbes may function as a potential source in the transportation of antimicrobial resistance to human pathogens[11,12]. At butchery/ slaughter, resistant strains from the gut readily contaminate poultry carcasses which often cause contamination of poultry meats and eggs during lay with multi resistant *E. coli*[13-15].

Due to enormous exploitation of antibiotics in the field of veterinary medicine, an increased number of resistant bacterial strains were developed in recent years. The transmission of plasmid mediated resistance between different bacterial species and genera are now widely occurred[16]. In different parts of the world, multi drug resistant strains of *E. coli* are ubiquitous in both human and animal isolates[17] and multiple drug resistant, nonpathogenic *E. coli* found in the intestine is probably an important reservoir of resistance genes[18] and momentarily drug-resistant *E. coli* of animal origin may colonize the human intestine[19]. Acquired multi drug resistance to antimicrobial agents creates an extensive trouble in case of the management of intra and extra intestinal infections caused by *E. coli*, which are a major source of illness, death, and increased healthcare costs[20]. Therefore, the present study was designed to isolate *E. coli* strains from five different sources of poultry and poultry environment of Bangladesh for assessing their susceptibility and resistance patterns to some selected antimicrobials.

MATERIALS AND METHODS

Sampling sites: A total of 250 samples were collected from Cloacal swabs of chicken, intestinal fluid of chicken, egg surface, faecal material of chicken and hand wash of chicken handlers from different poultry markets in Dhaka, Bangladesh.

Sampling from cloacal swab: Sterile swab stick moistened with sterile normal saline water was inserted in the cloacae of the chicken and placed in sterile vials.

Sample collection from intestinal fluid: The intestines were collected just after the sacrifice of chickens. Each intestine was placed separately into a sterile jar containing 500 mL of normal saline, and this suspended fluid of normal saline was used later for bacteriological analysis.

Sample of egg surface: 10 eggs collected from poultry cases just after laying were washed in 1000 mL of normal saline water and then taken into a sterile jar.

Collection of sample from faecal material: About 50 gm of fresh faecal sample was collected aseptically from poultry cases into sterile vials with the help of sterile cotton bud and 5 gm sample was transferred immediately to screw caped test tubes containing 10 mL of sterile nutrient broth.

Sample from hand wash of chicken handlers: Hands of the chicken handlers just after processing of slaughtered chickens and handling of chicken for sale were washed directly with 1000 mL of normal saline water and then taken into a sterile jar and sealed.

Transportation of sample: After collection, all the samples were transported to the laboratory immediately in an insulating foam box with ice.

Bacteriological analysis: A loop full of selective enriched broth from previously incubated sample from cloacal swab and faecal material and 0.1 mL of sample from intestinal fluid were spread on the solid surface of Eosine Methylene Blue (EMB) agar medium (Hi-Media, India), 1.0 mL sample from intestinal fluid was placed onto sterile plates which was then mixed with sterile medium (EMB) poured into the plates after being cooled to about 42-45 ^{o}C. 10-100 mL sample from egg surface and hand wash of chicken handlers was filtrated through the membrane filter (0.45 µm, Millipore, USA) which was then placed on the surface of EMB agar plates. All samples were incubated for 24 h at 37 ^{o}C in three triplications of EMB plates or filters on EMB agar for successful isolation of typical colonies. Identification was done according to Buchanan and Gibbons[21] following a series of biochemical tests included gram staining, tests for oxidase, methyl red, Voges-Proskauer reactions, indole, citrate, catalase, urea hydrolysis, gelatin hydrolysis, lactose fermentation, nitrate reduction, casein hydrolysis and sugar fermentation. Moreover, identification of *E. coli* was further confirmed by latex agglutination tests using polyvalent antisera (DENKA SEIKEN Co. Ltd, Tokyo, Japan).

Drug sensitivity test: Single disc diffusion method[22] was used to examine bacterial susceptibility to antimicrobial agents. A total of 13 antibiotic discs (Becton Dickinson, U.S.A.) with Streptomycin 10 µg, Erythromycin 15 µg, Chloramphenicol 30µg, Ciprofloxacin 5µg, Tetracycline 30µg, Penicillin 10 µg, Norfloxacin 10µg, Riphampicin 5µg, Neomycin 30µg, Cefixine 5µg, Ampicillin 10 µg, Kanamycin 20 µg and Gentamicin 10µg were used. By the standard method of inoculation, the top of a single and well-isolated colony

was touched with a sterile loop and the growth was inoculated into 2 mL of Mueller-Hinton broth. The broth culture was then allowed to incubate at 37 °C for 4 hours to obtain the young culture. The turbidity of actively growing broth cultures was then adjusted to a 0.5 McFarland standard and then a sterile cotton swab was dipped into the adjusted suspension within 15 min and excess broth was purged by pressing and rotating the swab firmly against the inside of the tube above the fluid level. The swab was then spread evenly over the entire surface of the plate of LB agar to obtain uniform inoculums. The plates were then allowed to dry for 3-5 min. Antibiotics impregnated discs were then applied to the surface of the inoculated plates with sterile forceps. Each disc was gently pressed down onto the agar to ensure complete contact with the agar surface. Even distribution of discs and minimum distance of 24 mM from center to center were ensured. Five discs (four antibiotics discs and one blank disc as control) were placed in each petri dish. Within 15 min of the application of the discs, the plates were inverted and incubated at 37 °C. After 16-18 h of incubation, the plates were examined, and the diameters of the zones of complete inhibition to the nearest whole millimeter were measured. The zone diameter for individual antimicrobial agents was then translated into susceptible, intermediate and resistant categories according to the interpretation table of the Becton Dickinson Microbiology Company, USA.

RESULTS

Among the *Escherichia coli* strains isolated from poultry and poultry environment, a total of 80 were selected and subjected to various morphological and biochemical tests followed by serological identification. The distribution pattern and the biochemical tests for identification of *E. coli* isolates from poultry sources are summarized in Table 1 and 2, respectively. 58 % of total samples were found *E. coli* positive. The incidence range of all 5 types of sample sources found was from 42 % in egg surfaces to 82 % in feces.

Antibiotic susceptibility pattern of *E. coli* isolates from samples of poultry sources has been outlined in Table 3. Resistance spectrum of *E. coli* for 13 antibiotics tested in descending order was respectively Penicillin, Ciprofloxacin, Riphampicin, Kanamycin, Streptomycin, Cefixine, Erythromycin, Ampicillin, Tetracycline, and Chloramphenicol and Neomycin, with ranges of percent strains resistant from 20 % in case of neomycin and 88 % for penicillin (Table 3). No strain was found either sensitive to erythromycin,

Table 1: Distribution of *Escherichia coli* in various samples from poultry and poultry environments of Bangladesh

Sample source	No. of samples tested	No. of samples positive for *E. Coli* detection	Percentage positive samples
Cloaceal Swab	50	33	66
Intestinal Fluid	50	27	54
Egg Surface	50	21	42
Faecal material	50	41	82
Hand Wash of Chicken Handler	50	23	46
Total	250	145	58

Table 2: Biochemical tests used for identification of *Escherichia coli*

Name of tests	*Escherichia coli* reaction	% of isolates with same reaction as *E. coli*
Gram Staining	G^-, Small Rod, Pink	99
EMB	BCMS	99
Citrate Test	-	80
Oxidase Test	-	75
Indole Test	+	75
Methyle Red Test	+	75
Voges-Proskauer	-	60
Reactions		
Sugar Fermentation	+	90
Catalase Test	+	65
Lactose Test	+	90
Urea Hydrolysis test	+	75
Nitrate Reduction Test	+	80
Gelatin Hydrolysis Test	+	75
Casein Hydrolysis test	+	65

G^- = gram negative; BCMS = black centered colony with metallic sheen; + = 90 to 100 % of the isolates were positive; − = 0 to 10 % of the isolates were positive.

riphampicin, kanamycin, cefixine, penicillin and ciprofloxacin or resistant to gentamycin and norfloxacin (Table 3). Moreover, 12-36 % strains were found intermediate resistant to 11 antibiotics out of total 13 tested. All 50 isolates examined in this study showed multiple resistances to at least 6 up to 10 antibiotics. The highest sensitivity was recorded for the antibiotic norfloxacin in 86% of the strains tested and it was followed respectively by chloramphenicol and gentamycin 80 %, neomycin 60 %, tetracycline 36 %, streptomycin 30 % and ampicillin 26 %.

DISCUSSION

The prevalence of *E. coli* in 82 % of fecal samples in the present study was higher than the previous records of Rahman *et al.*[23]. The egg surface was contaminated with *E. coli* probably from poultry feeds and/ with feces during lay in unhygienic condition or also from infected poultry. Among the animal protein ingredients, a major ingredient of poultry feeds, locally

Table 3: Antibiotic susceptibility pattern of 50 selected strains of *Escherichia coli*

Antibiotics	Sensitivity groups of *Escherichia coli* isolates					
	Resistant		Intermediate		Sensitive	
	% of strains positive	Inhibition zone (mm)	% of strains positive	Inhibition zone (mm)	% of strains positive	Inhibition zone (mm)
Chloramphenicol (30µg)	20.00	<25	00.00	26-28	80.00	>29
Erythromycin (15µg)	64.00	<15	36.00	16-20	--	>21
Ampicillin (10µg)	58.00	<13	16.00	14-15	26.00	>17
Gentamicin (10µg)	--	<06	20.00	7-9	80.00	>10
Riphampicin (5µg)	80.00	<15	20.00	17-19	--	>20
Neomycin (30µg)	20.00	<12	20.00	13-14	60.00	>15
Kanamycin (20µg)	76.00	<13	24.00	14-17	--	>18
Cefixine (5µg)	68.00	<14	32.00	14-15	--	>18
Penicillin (10µg)	88.00	<28	12.00	NA	--	>29
Tetracycline (30µg)	52.00	<25	12.00	26-28	36.00	>29
Streptomycin (10µg)	70.00	<06	00.00	7-9	30.00	>09
Norfloxacin (10 µg)	--	<12	14.00	13-15	86.00	>17
Ciprofloxacin (5 µg)	82.00	<30	18.00	30-33	--	>33

processed cheap fish wastes were found to be important causes for bacterial contamination of poultry feeds[24]. *E. coli* was reported as a common microflora in raw feeding materials and poultry feeds[25]. Present study showed a high percentage of egg surface samples 42 % contained *E. coli*. The pre-stuffed chickens in poultry shops, poultry and poultry products like eggs and plastic-wrapped poultry meat in various super shops get contaminated easily by *E. coli* for the careless unhygienic handling process and ready-to-eat foods become cross contaminated with *E. coli* as well as other pathogenic bacteria from food handlers with poor personal hygiene and from other raw poultry products.

Resistance of *E. coli* isolates from Malaysian broiler chicken to ampicillin, tetracycline and gentamicin with 11-95 % range has been reported[26]. Rahman *et al*.[23] reported *E. coli* isolates from broiler and layer poultry in Bangladesh were found resistant to chloramphenicol, ampicillin, ciprofloxacin, tetracycline and streptomycin in 37-87.5 % cases; and 50-66.6 % strains highly sensitive to chloramphenicol and gentamicin. 66-100 % *E. coli* strains from poultry in Bangladesh showed resistance to tetracycline, penicillin, erythromycin and chloramphenicol[27]. Tricia *et al*.[28] reported 43 % isolates of *E. coli* were resistant to ampicillin but no isolate was found resistant to gentamicin, which is in agreement with this present study. Daini and Adesemowo[29] found the resistance of *E. coli* from Nigeria in 54 and 88 % strains against gentamicin and tetracycline respectively.

All the isolates of present study exhibited multiple resistances to more than six antibiotics. Similar findings on multiple drug resistance of *E. coli* strains has been reported from Bangladesh and other parts of the world[23,30-32]. Due to indiscriminate exploitation of antimicrobial agents, such high incidence of multi drug resistance may apparently be occurred which may ultimately replace the drug sensitive microorganisms from antibiotic saturated environment[11].

Reduction in the frequency of vancomycine resistant *Enterococci* from broilers from 80 % to 5 % due to ban imposed on avoparcin as a feed additive for poultry in Denmark[33] justifies encountering this resistance emergence with reduced and judicious application of antibiotics in animal farming and clinical purposes.

CONCLUSION

Risk assessment should reflect the increasing weight of scientific evidence indicating the potential for even non-pathogens carrying and transferring genetic determinants for antibiotics resistance to human pathogens, cross-resistance development, and potential link between resistance to critical antibiotics in human medicine and use of similar drugs in poultry feeds. Appropriate use of antibiotics in humans and farm animals needs to be addressed in Bangladesh and other countries.

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