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Prevalence of antimicrobial resistance of *Escherichia coli* isolated from broiler at Rajshahi region, Bangladesh

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Abstract

Antimicrobial resistance, a major, health problem for both humans and animals throughout the world, is leading to treatment failure after administering antimicrobial drugs. This study was conducted from December 2012 to May 2013 to determine the prevalence of antimicrobial resistance of Escherichia coli in live broilers in Rajshahi district of Bangladesh. Five Upazilla were selected randomly and 200 cloacal swab samples of live birds were collected from 50 different broiler farms (four samples from each farm). A flock was classified as resistant to antimicrobials if one of the four samples showed resistance to any of the nine antimicrobials tested for. Isolation of E. coli was done by conventional microbiological methods followed by biochemical identification.

A total of 200 E. coli isolates were collected and tested for resistance to nine antimicrobial agents (ampicillin, erythromycin, tetracycline, gentamicin, ciprofloxacin, levofloxacin, trimethoprim-sulfamethoxazole, colistin sulphate, and streptomycin). Antimicrobial resistance tests were performed by following the standard disc diffusion technique mentioned by the Clinical and Laboratory Standards Institute (CLSI-2011). Results showed that all isolates are multi-drug resistant (≥5 antimicrobial agents) and all were 100% resistantto tetracycline, erythromycin, streptomycin, ampicillin, trimethoprim sulphamethoxazole and ciprofloxacin. The highest sensitivity pattern of 73.5% of the isolates was determined for colistin sulphate followed by gentamycin (49%), and levofloxacin (17%). Results suggest that a high resistance of E. coli to antimicrobials exerts a threat to the poultry industry at Rajshahi area in Bangladesh, so raising awareness about proper administration of antimicrobials in broiler farms is crucial from an animal production and veterinary public health point of view.

Keywords: E. coli, Antimicrobials, Resistance, Broiler, Bangladesh

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1. Introduction

Antimicrobial resistance is becoming a global threat all over the world and due to the rapid increasing nature of this problem; the World Health Organization (WHO) recommended a global surveillance system in veterinary and human medicine [1]. The theme of the World Health Day 2011 proposed by WHO was "Antibiotic resistance: No action today, no cure tomorrow" and it was selected to create mass awareness among the world population. Anti-microbial abuse is considered to be the most vital selecting force to antimicrobial resistance of bacteria [2, 3].

Escherichia coli, a common microbial inhabitant of gastrointestinal tract of poultry and human being including other animals but may become pathogenic to both [4, 5]. Most of the E. coli strains are nonpathogenic and may serve as indicator of fecal contamination in food. About 10-15 % of intestinal coliforms are opportunistic and pathogenic in nature [6]. Pathogenic E. coli can cause a variety of lesions in immunocompromised hosts and in poultry. Diseases caused by E. coli are sometimes severe. Sometimes even lethal such as meningitis, endocarditis, urinary tract infection, septicemia, epidemic diarrhea of adults and children [7] and yolk sac infection, omphalitis, cellulitis, swollen head syndrome, coligranuloma, and colibacillosis in poultry [8].

Antimicrobial treatment is considered the most important issue that promotes the emergence, selection and spread of antimicrobial resistant microorganisms in both veterinary and human medicine [9, 10]. Many studies have shown the spread of antimicrobial resistance from animals to humans [11-15]. Acquired multi drug resistance to antimicrobial agents may lead to illness, death, and increased healthcare costs if it occurs due to treatment of infections caused by E. coli [16].
Bangladesh has a large amount of poultry production. In 2011, there were over 115,000 farms producing approximately 170 million broilers and layers [17]. There is no detailed information on antimicrobial resistance in animals or humans ecospheres in Rajshahi area. Hence, the present study was designed to isolate E. coli strains from fifty different broiler farms of Rajshahi district in Bangladesh for assessing their susceptibility and resistance patterns to some selected antimicrobials.

2. Material and Method

2.1. Study design
The study is a cross-sectional survey conducted from December 2012 to May 2013.

2.2. Study site
Rajshahi is a divisional city as well as a big district with a population of 2.2 million. It is located in the western part of Bangladesh bordering India to the west. It plays an important role in the food industry as the number of households involved in animal food production was 388767 [18]. Out of these, 660 broiler farms produced 1,980,269 broilers in 2011 [19]. Products are both locally consumed and exported to nearby districts. Antimicrobial use has been reported to be huge (Survey data). Major types of antimicrobials include ciprofloxacin, enrofloxacin, colistin sulphate, doxycycline and levofloxacin.

2.3. Sampling technique
From the list of broiler farms available at the Rajshahi district Livestock Office [19], 50 farms were selected by multistage random sampling method. Selection criteria included: at least 500 broilers in the farm, safety from political unrest and accessibility by paved road. Four broilers were randomly selected from the same farm and cloacal swabs were carried out. All swabs were kept in transport media and sent to the laboratory as soon as possible on the same day.

2.4. E. coli isolation

On the day of arrival at the laboratory, the cloacal swabs from the broilers were streaked on Mac Conkey agar (Merck) and incubated aerobically at 37°C for 24 hours. Lactose fermenting colonies were then streaked on Eosin Methylene Blue (Merck) agar and incubated for 24 hours at 37°C. Colonies that produce greenish metallic sheen were isolated on Nutrient Agar (Merck) [20, 21]. From the Nutrient agar E. coli isolates were inoculated in half-strength Nutrient Agar (Merck) (incubated at 37° C for 24 hours) and stored at 4°C for further identification.

2.5. Biochemical identification

Suspected colonies were confirmed as E. coli by negative gram's stained rods with positive glucose/lactose fermentation, gas production and absence of H2S production in Triple Sugar Iron agar (Merck), positive Methyl Red test and negative Vogues Proskauer test in Methyl Red Voges Proskauer media (Merck), Indole production in Motility Indole Lysine media (Oxoid), and negative reaction in Urea Agar (Himedia) and Simmon's Citrate agar (Oxoid) [22]. Besides E. coli confirmatory test (EC), gas production in Durham tube and Trypton Water (TW) test, red ring after adding covac's reagent were also done for further identification.

2.6. Antimicrobial resistance testing

Antimicrobial resistance tests were performed by standard disc diffusion technique (CLSI-2011). The selection criteria of antibiotics testing discs depended on the regularly use of antimicrobials in the broiler farms, potential public health importance and recommended from the guideline of antimicrobial susceptibility testing from CLSI (2011). Resistance testing discs contained ampicillin (10 µg), colistin (10 µg), gentamicin (10 µg), streptomycin (10 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), erythromycin (15 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), tetracycline (30 µg) (Oxoid). The isolates were considered resistant if the diameter of inhibition zone was less than or equal to the resistance breakpoint provided by CLSI guidelines. Quality control of diameters of inhibition zone against the level of resistance was based on E. coli (ATCC-25922) as a reference.

2.7. Data management and statistical analysis

Data processing has done by computer using Epidata version 1.3.2.1 (The Epidata Association, Odense, Denmark) and R software version 3.0.1 (R, Free Foundation for Statistical Computing, Boston, USA).

3. Results

3.1. Number of E. coli isolates

Among 200 specimens collected from 50 different farms all were found E. coli positive (Table-1). From all positive samples we collected 200 isolates for further analysis and preserved them in half strength nutrient agar at 4°C. Of the isolates, 100% were resistant to six antimicrobials named ampicillin, tetracycline, streptomycin, ciprofloxacin, erythromycin and trimethoprim-sulphamethoxazole. The highest sensitivity showed by colistin sulphate (73.5%), followed by gentamycin (49%) and levofloxacin (17%) respectively (Table- 2).

Table. 1. Sources and characteristics of samples

No.	*Name of upazilla*	*No of farms from where samples taken*	*No. of samples taken*	*No. of samples in which E. coli were recovered*	*No. of E. coli isolates*
1	Durgapur	09	36	36	36
2	Sadar	09	36	36	36
3	Godagari	09	36	36	36
4	Mohanpur	15	60	60	60
5	Tanore	08	32	32	32
	Total	50	200	200	200

Table. 2. The sensitivity, intermediate resistance and resistance of E. coli isolates against the tested antimicrobials.

Name of Agents	Susceptible		Intermediate		Resistance	
	n	%	n	%	n	%
Colistin	147	73.5	0	0	53	26.5
Gentamycin	98	49	30	15	72	36
Levofloxacin	34	17	36	18	130	65
Ciprofloxacin	0	0	64	32	136	68
Ampicillin	0	0	0	0	200	100
Streptomycin	0	0	0	0	200	100
Erythromycin	0	0	0	0	200	100
Trimethoprim + Sulphamethoxazole	0	0	0	0	200	100
Tetracycline	0	0	0	0	200	100

There were several resistant patterns and the major resistant patterns are showed in the table 3.

Table. 3. Major resistance Patterns of E. coli isolates (N=200)

Multi-Drug Resistance Pattern	(n)	(%)
AMP+STR+ ERY+SXT+TE + LEV+CIP	54	27
AMP+STR+ ERY+SXT+TE + LEV+CIP +CN	22	11
AMP+STR +ERY+SXT+TE+CIP	19	9.5
AMP+STR +ERY+SXT+TE+LEV	13	6.5
AMP+STR+ ERY+SXT+TE + COL+LEV+CIP	13	6.5
AMP+STR+ERY+SXT+TE	11	5.5
AMP+STR+ERY+SXT+TE +CN+CIP	11	5.5
AMP+CN+STR+LE+ERY+SXT+TE	9	4.5

4. Discussion

The prevalence of E. coli in 100 % of cloacal swab samples in the present study was higher than the previous records of Akond et al., [23] in Bangladesh, which were 66% only. Our study clearly indicates a higher resistance rate than previous study by others.

Many studies on antimicrobial resistance were performed in Bangladesh at different times in different region but none in Rajshahi area. M. Hossain et al., [24] from Bangladesh reported 91.42% isolates were resistant to erythromycin and 62.85% to ampicillin but in our study all isolates are resistant to both of these drugs. In contrast to our study, Nazir et al., [25] reported higher sensitivity to ciprofloxacin. In another study conducted in Bangladesh by Hashem et al., [26] claimed 100% sensitivity to colistin sulphate and here it is only 73.5% indicating an abuse of colistin.

Our results support the study by Al-Ghamdi et al., [27] who showed (99.1%) resistance to tetracycline in Saudi Arabia. All isolates of this present study exhibited multiple resistances to more than six antibiotics. Similar findings on multiple drug resistance of E. coli strains have been reported from Bangladesh and other parts of the world [11, 27, 28].

The higher resistant rate may be due to an indiscriminate use of Antimicrobials. 80% (40/50) farmers used antimicrobials as preventive treatment. Due to the direct influence of dealer (Chick, feed and drug seller) farmers are practicing indiscriminate administration of antimicrobials. Ciprofloxacin, enrofloxacin, colistin sulphate, doxycycline, trimethoprim + sulphamethoxazole, levofloxacin are the major antimicrobials used by the farmer (Based on survey). Antimicrobials are available and can be purchased without prescription from a veterinarian. Due to indiscriminate exploitation of antimicrobial agents, high incidence of multi drug resistance may ultimately replace drug sensitive microorganisms from antimicrobial saturated environments [29].

5. Conclusions

The study suggests that the prevalence of resistant E. coli from live broiler is quite significant in respect of indiscriminate use of antimicrobial drugs. A comprehensive antimicrobial drug administration monitoring system should be urgently devised and implemented.

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Conflicts of interest

The authors declare no conflicts of interest.

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