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Research article

Isolation, O-serotypic characterization and *in-vitro* susceptibility of *Escherichia coli* isolates from chickens to five common antimicrobials used in Bangladesh

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A B S T R A C T

A total 1043 dead chickens were collected from different chicken farms located in Chittagong district, Bangladesh; all these dead chickens were examined postmortem. In total, 162 broiler and 183 layer chickens had lesions consisting fibrinous peri-hepatitis; of them, on bacteriological examination, 88 and 82, respectively, were positive for *Escherichia coli*. Serological analysis revealed that 19, 24, 26 and 48 of the isolates belonged to serotypes O1, O2, O8 and O78, respectively; 53 isolates remained "untypable." By disc-diffusion method isolates were tested for their susceptibility to 5 antimicrobials: amoxicillin, cephalexin, co-trimoxazole (sulphamethoxazole/trimethoprim), gentamicin and oxytetracycline. Most of the *E. coli* isolates of broiler and layer chicken origins were resistant to co-trimoxazole (86.4 *vs* 91.5) and oxytetracycline (56.8 *vs* 78.1) followed by amoxicillin (47.7 *vs* 73.2); <16 % isolates from both layer and broiler chickens were resistant to gentamicin; however, 34% broiler isolates were resistant to cephalexin.

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INTRODUCTION

The term colibacillosis refers to any localized or systemic infection produced solely or partly by *Escherichia coli* (*E. coli*) (Calnek, 1997). Collectively, infections caused by *E. coli* are responsible for significant economic losses to both broiler and layer chicken farms (Yogaratnam, 1995).The intensity of pathogenicity caused by *E. coli* is based on the serotype (s) involved in the process (Biswas *et al*., 1996; Redchuk, 1974). In different parts of the world, over many years, the most common serotypes of *E. coli* affecting commercial chickens have been O1, O2, O8, O9, O35 and O78 (Biswas *et al*., 1996; Redchuk, 1974; Sojka, 1965; Heller and Drabkin, 1977; Lior, 1994; Wray and Woodward, 1994; Wray and Davies, 1996). To combat bacterial diseases in commercial chickens in Bangladesh antibiotics are being used for both therapeutic and

prophylactic applications. Because the country has not got any monitoring system on the rational usages of antibiotics, the current promiscuous type of antimicrobial using might result in the emergence of multi-drug-resistant strains of pathogenic bacteria (Mateu *et al*., 2002). The degree of resistance acquired by *E. coli* might be transferred to the closely related organisms by the plasmid (Gross, 1994). The aims of the present study were to isolate the serotypes of *E. coli* affecting both broiler and layer chickens reared on commercial chicken farms in Chittagong district, Bangladesh and assess susceptibility of the isolates to five antimicrobials commonly used in therapeutic or prophylactic mass-medication against bacterial diseases in the country.

MATERIALS AND METHODS

Minimum sample size required for the investigation

The two production categories of chickens-broiler and layer, were regarded as two populations for sampling. The required sample size per production category was 76 based on the formula, $n = 4pq/l^2$ (Farmer and Kelly, 1991). Because the prevalence of *E. coli* infection/colibacllosis in both the chicken types was initially found to be ≤ 5%, a prevalence of 5% was considered with a precision of 5% to reckon the minimum sample required for each production category.

Collection of dead chickens

Dead chickens from different layer and poultry farms in the district of Chittagong were collected and investigated. A part-time field assistant was employed to collect dead chickens and to get them submitted to the department of Microbiology, Chittagong Government Veterinary College (the former name of Chittagong Veterinary and Animal Sciences University), Bangladesh. A routine post-mortem examination was done on each collected chicken, and a possible case of colibacillosis was suspected having fibrinous perihepatitis (Wray and Davies, 1996) on this examination.

Isolation and identification of *E. coli*

For each carcass with a lesion of fibrinous peri-hepatitis bacteriological inocula were taken from three organs—bone marrow, heart and liver by 3 sterile cotton swabs. With these inocula taken primary inoculation was made on MacConkey agar surface. A presumptive diagnosis of *E. coli* growth on this agar plate was determined by finding large bright pink color colonies produced on it after 48 hours of incubation at 37°C. When the said characteristic colonies developed from inocula taken from at least 2 of the 3 organs, then the colonies were further analyzed to characterize the organism (Redchuk, 1974). To initiate the identification process, a typical single discrete colony was picked up, inoculated into nutrient broth and incubated at 37^0C for 24 hours. The broth culture, thus achieved, was used to conduct various biochemical tests for identification of *E. coli* based on the method described by Farmer and Kelly (1991).

Serotyping of *E. coli* isolates

O-serotypic characterization of an *E. coli* isolate was made by performing plate agglutination test with the boiled *E. coli* culture to 4 mono-valent O-colisera (O1, O2, O8, O78) as per the Manufacturer's instruction (Statens Serum Institut, Copenhagen, Denmark).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed on Mueller-Hinton agar (Difco Laboratories) by the Kirby-Bauer micro-disc diffusion technique according to the United States National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999). To test the susceptibility five antimicrobial compound disks, manufactured by Oxoid Company, UK were used; they were amoxicillin, cephalexin, cotrimoxazole (sulphamethoxazole/trimethoprim), gentamicin and oxytetracycline with disc potency of 15,30, 25 (23.75/1.25), 10 and 30 µg, respectively. Measurement of the growth inhibition zones allowed the classification of each isolate as susceptible, intermediate or resistant according to the NCCLS (1999).

Statistical analysis

The significance of difference in the occurrence of *E. coli* between the collected broiler and layer chickens was shown using a χ^2 test.

RESULTS

In total, 1043 dead chickens were examined post-mortem having collected them from different farms. The numbers of broiler and layer chickens that had fibrinous peri-hepatitis during post-mortem examinations are shown in Table 1; of them 88 and

82 chickens of the broiler and the layer production category, respectively were positive for *E. coli*. There was no significant difference on the occurrence of *E. coli* in broiler chickens compared with layer (P>0.05). Of the 170 *E. coli* isolates 117 belonged to any of the four O-sero-groups--O1, O2, O8 and O78; other 53 (30.6%) isolates remained "untypable". Among the O-serotypes O78 was the predominant one (Table 1).

Table 1. Frequencies of 4 O-serotypes of *Escherichia coli* in dead chickens from Bangladesh (1043 chickens; January-June, 2001)

Production type	N	n^+ (%)	Frequency				
			O1	O2	O8	O78	Untypable
Broiler	162	88 (54.3)	9	18	15	27	19
Layer	183	82 (44.8)*	10	6	11	21	34
Total	345	170 (49.3)	19	24	26	48	53

N= No. dead chickens that all had peri-hepatitis lesions; n^+= No. positive for *E. coli*; * P>0.05

Table 2. Antimicrobials susceptibility of 170 *Escherichia coli* isolates of chicken origin from Bangladesh to 5 selected antimicrobial agents

Antimicrobial agent (disc potency)	Antimicrobial susceptibility results (%)				Break point (mm)*	
	Susceptible		Intermediate		Resistant	
	IoLO	IoBO	IoLO	IoBO	IoLO	IoBO
Amoxicillin (25 µg)	12.2 (≥18)	10.2	14.6 (14-17)	42.1	73.2 (≤3)	47.7
Cephalexin (30 µg)	66.1 (≥18)	54.6	17.1 (15-17)	11.4	15.9 (≤14)	34.1
Co-trimoxazole (23.75/1.25 µg)	2.4 (≥16)	3.4	6.1 (11-15)	10.2	91.5 (≤10)	86.4
Gentamicin (10 µg)	75.6 (≥15)	75.0	14.6 (13-14)	11.4	9.8 (≤12)	13.6
Oxytetracyline (30 µg)	9.2 (≥19)	26.1	12.2 (15-18)	17.1	78.1 (≤14)	56.8

*Break points--growth inhibition diameters that define a given category; IoLO=Isolate of layer origin; IoBO=Isolate of broiler origin

The antimicrobial compounds for which a lower proportion of resistant isolates recognized was gentamicin followed by cephalexin with 9.8 and 15.9%, respectively (Table 2).

Most of these isolates were resistant to co-trimoxazole, whereas, 78.1% isolates showed resistance to oxytetracycline. Of the 82 layer isolates 73.2% showed resistance to amoxicillin. Like layer isolates broiler isolates of *E. coli* showed the highest proportionate resistance to co-trimoxazole followed by the second highest to oxytetracycline. The lowest proportion of broiler isolates, which was 13.6%, was resistant to gentamicin, where as 34.1% *E. coli* isolates of this production category showed resistance to cephalexin.

DISCUSSION

There are so far 173 O-serotypes of *E. coli* reported (Lior, 1994; Wray and Woodward, 1994; Wray and Davies, 1996), of which O1, O2, O8, O9, O35 and O78 are the common pathogenic serotypes unraveled from chickens. Due to short falling of resources, we used only 4 mono-valent coli sera (O1, O2, O8 and O78). In this study, the isolates, which were not sero-typed, were categorized as "untypable" - some or all of them might have belonged to other serotypes. However, to the authors' knowledge, this study could be the first one that identified at least 4 O-serotypes of *E. coli* affecting chickens in Bangladesh. In Bangladesh, *E. coli* isolates of chicken origin said to be susceptible to the 5 antimicrobials tested were simply rare. Categorization as intermediately sensitive to a given

antimicrobial was considered as a decreased susceptibility in order to avoid overestimation of the rate of resistance. Nevertheless, more than 50% isolates of *E. coli* of broiler and layer origins were resistant against oxytetracycline and cotrimoxazole. The probable explanation of the high rate of occurrence of resistant isolates of *E. coli* to cotrimoxazole and oxytetracycline is that, in Bangladesh, these two drugs are being used in therapeutics and chemoprophylaxis for a longtime. In addition, a very common fashion observed in the country is that the farm-owners are used to keeping a collection of previously dispend but incompletely used antibiotics, which they use later in chickens without seeking any veterinary attention. Also in the country there are frequent instances that the farmers use these two drugs simultaneously or even the same agent with two different trade names based on their own choices or having advised from the feed sellers. Such a promiscuous type of antimicrobial using might have led the emergence of resistant isolates of *E. coli* to cotrimoxazole and oxytetracycline. Resistance to broad spectrum penicillin group like amoxicillin was also found with an increasing rate as compared with other reports in different countries (Bensink and Bothmann, 1991; Mishra, 1991). The rate of resistance to gentamicin in the present study remained lower than Babila and Akcadag (1992) who found the rate 23%. The probable explanation on why the *E. coli* isolates had a decrease resistance to gentamicin is that, in veterinary field in Bangladesh, the drug has recently been introduced, and the farmers do not like to choose the drug to treat their chickens because it is administered parentally.

Surprisingly, a great percentage of isolates of broiler origin were resistant against cephalexin, which has been got very limited usages in veterinary field in Bangladesh. There is no definitive explanation for the resistance of a significant number of isolates to cephalexin but it might indicate the probable use of cephalexin for human preparation in the treatment of bacterial diseases in chickens. Antimicrobial resistance of bacteria emerged as consequences of veterinary applications are currently one of the key issues in public health debates (Mateu *et al*., 2002; WHO, 2000). The results in the present study indicate that antimicrobial resistance is a real problem in *E. coli* affecting chickens in Bangladesh which might have been developed due to their excessive usages. These facts should alert farm owners, veterinarians and public health authorities to generate the awareness that antibiotics, if required, should be used with extreme caution and rationale for the treatment of chicken diseases.

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