



Isolation of *Escherichia coli* from the Liver and Yolk Sac of Day Old Chicks with their Antibiogram

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Abstract

This study aimed at isolation of *Escherichia coli* (*E. coli*) from liver and yolk sac of day old chicks from M. M. Agha Poultry, Chittagong, Bangladesh. A total of 100 samples were tested during July to August, 2015 where 47 (47%) samples were positive for *E. coli* through all classical bacteriological tests. By using disc diffusion method isolated *E. coli* were tested for resistance against 8 different antimicrobial agents. *E. coli* exhibited 100% resistance to Tetracycline, followed by Amoxicillin (82.98%) and Ampicillin (85.11%). Conversely, 100% sensitivity was observed in Gentamicin and Ceftriaxone which was followed accordingly Ciprofloxacin (44.68%), Colistin Sulphate (57.45%) and Enrofloxacin (59.58%). Intermediate zone of sensitivity was observed in Enrofloxacin (25.53%) Ciprofloxacin (29.79%) and Colistin Sulphate (29.79%). Every isolates showed resistances against multiple antimicrobial agents. So, it is important to monitor the occurrence of antimicrobial resistance in day old chicks which is very essential for public health context.

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1. Introduction

Avian Colibacillosis is one of the most infectious disease of all ages of poultry which results a variety of disease conditions including yolk sac infection, omphalitis, swollen head syndrome, septicemia, enteritis, salpingitis and respiratory tract infection. The acute form of Colibacillosis in poultry is characterized by septicemia resulting in death whereas pericarditis, airsacculitis and peri-hepatitis indicate the subacute form (Calnek et al., 1997). During the first week of post-hatching of broilers, yolk sac infection (YSI) may cause a high mortality (Bains, 1979). Colibacillosis affected bird's show depression, growth retardation and respiratory distress where usually morbidity is more than 50% but Mortality is below 5% (Wray et al., 1996;

Vandekerckhove et al., 2004). Yolk sac is the main source of infection for Day Old Chicks while the older chicks are infected via respiratory route. Broiler chickens below three weeks of age are highly susceptible to primary colibacillosis but four weeks or older chicks have quite resistance against it (Goren, 1978). However, various risk factors may have synergistic effect to the susceptibility to colibacillosis in poultry. In addition, the concentration of *E. coli* in the the broiler house environment acts as an important factor (Harry and Hemsley, 1965).

Now a days, microbiological food safety has become an important public health issue worldwide .In the gastrointestinal tract of poultry and human being *E. coli* is one of the most common microfloras (Jawetz et al., 1984). Though most of the *E. coli* are not pathogenic but they indicates contamination in food by fecal materials and about 10 to 100% of intestinal coliforms are found as opportunistic and pathogenic serotypes of *E. coli* (Barnes et al., 1997). Several studies and research have established animal originated foods as principal vehicles associated with disease condition caused by *Yersinia*, *Salmonella* and *E. coli* spp. (Cretikos et al., 2008).

At present, for different purposes such as prophylaxis, growth promotion or therapeutics, antibiotics have been used successfully in poultry industries (Castanon, 2007). Moreover, in both veterinary and human medicine, recklessly use of antibiotic is considered as the major issue for developing resistant microorganisms (Neu, 1992). Many well-established evidence proved that treatment by antibiotics can lead to the development and dissemination of resistant *E. coli* which can then be transferred into people through food or direct close contact with sick animals. These resistant microbial organisms may act as a potential source in the transmission of antimicrobial resistance to human pathogenic organisms (Van de Bogaard et al., 2001; Schroeder et al., 2002). At abattoir, resistant strains from the gut can readily contaminate poultry meats as well as eggs during lay with multi resistant *E. coli* which is very threatening (Turtur et al., 1990).

Due to reckless use of antibiotics in veterinary medicine sector, an increased number of multidrug resistant bacterial serotypes were developed over the last few years. The transmission of resistance among different bacterial species and genera are now widely happened by plasmid (Davies, 1994). Multi drug resistant strains of *E. coli* are developing in both human and animal isolates In different parts of the world, (Amara et al., 1995) and nonpathogenic *E. coli* having multiple drug resistance found in the intestine is an important carrier of resistance genes (Osterblad et al., 2000). Having resistance to different antimicrobial agents is an extensive trouble in case of the management and treatment of intra and extra intestinal infections caused by *E. coli*, which is responsible for illness, increased healthcare costs and death (Gupta et al., 2001). Therefore, the present study was designed to isolate *E. coli* strains from of day old chicks of Chittagong, Bangladesh for assessing their susceptibility and resistance patterns to some selected antimicrobials. Considering the above facts, present study was targeted to fulfill the following aims and objectives.

1. To isolate the *E. coli* from liver and yolk sac of Day Old Chick (DOC) of commercial broiler
2. To detect the antibiotic resistance patterns of *E. coli* against different antimicrobial widely used in poultry

2. Materials and Methods

2.1. Ethical statement

The study was conducted on Day Old Chicks (DOC) from a hatchery, M M Agha Ltd. at Chittagong, Bangladesh during the period of July to August 2015. A total number of 100 Day Old Chicks (DOC) were brought from above mentioned hatchery and tested at PRTC laboratory, Chittagong. Postmortem examination of the day old chicks was done by maintaining standard procedures and also taking all ethical means.

Sample was collected from liver and yolk sac by using sterile cotton swab and inoculated into test tube containing peptone broth. A loop full of selective enriched broth from previously incubated sample from liver

and yolk sac were spread on the solid surface of MacConkey agar. All samples were incubated for 24 hours at 37°C. Suspected colony was identified according to Buchanan and Gibbons (1974) and spread it over the solid surface of the Eosin Methylene Blue (EMB) agar, and it also incubated for 24 hours at 37°C. Bacterial colony identification was done according to following a series of biochemical tests included gram staining, and tests for indole.

Table 1: Methods on culture and biochemical test were done

Sl. No.	Agar/ test	Incubation time and temperature	Observations
1.	MacConkey agar	Incubated at 37°C temperature for 24 hours.	Dark pink colored raised colony
2.	Eosin Methylene Blue (EMB) agar	Incubated at 37°C temperature for 24 hours.	Characteristics Metallic sheen
3.	Indole test	Incubated at 37°C temperature for 48 hours then Kovac's indole reagent was added.	Red colored layer

Commercially available antimicrobial discs (OXOID, Hampshire, England) (CEF= Ceftriaxone; GEN= Gentamycin; CT= Colistinsulphate; AMP= Ampicillin; CIP= Ciprofloxacin; TEC= Tetracycline; ENR= Enrofloxacin; AMX= Amoxicillin.) were used to observe the antimicrobial sensitivity of *E. coli* by measuring the diameter of zone of inhibition. The antimicrobial sensitivity test of the isolated bacteria from the sample of day old chicks (DOC) were performed by using standard paper disc diffusion method was described by NCCLS (2009). For the interpretation of antimicrobial sensitivity by measuring the diameter of zone of inhibition following standard was followed:

Table 2: Standard measurement of diameter of zone of inhibition.

Sl. No.	Name of Antimicrobial agents	Diameter of zone of inhibition (millimeter)		
		Resistant	Intermediate	Sensitive
1	Ampicillin	≤13	14-16	≥17
2	Amoxicillin	≤13	14-17	≥18
3	Gentamycin	≤12	13-14	≥100
4	Ciprofloxacin	≤100	16-20	≥21
5	Enrofloxacin	≤16	17-19	≥20
6	Colistinsulphate	≤10	11-13	≥14
7	Tetracycline	≤11	12-14	≥100
8	Ceftriaxone	≤13	14-17	≥18

Source: CLSI, 2007; Seol et al., 2005; LO-Ten-Foe et al., 2007

Data were entered into MS excel (Microsoft Word 2007) and descriptive analysis was done in this study.

3. Results

Among 100 samples collected from liver and yolk sac of day old chicks (DOC), a total of 47 (47%) individual colonies of *E. coli* were isolated through different tests.

Results of cultural and biochemical examination

47% samples are positive in MacConkey which is also positive in EMB agar by producing metallic sheen. In the microscopic examination of Gram's staining, all the positive samples are found as Gram-negative, pink colored, rod shaped bacteria which are arranged in single or in pairs. Red colored ring also observed in all the agar positive samples which indicates indole positive.

Table 3: The examination result at a glance done for isolation of *E. coli*

Sl. No.	Name of the media /test	Total no. of sample	No. of positive sample	Percentage (%)
1.	MacConkey agar	100	46	46
2.	Eosin Methylene Blue (EMB) agar	100	47	47
3.	Gram 's staining	100	45	45
4.	Indole test	100	47	47

Result of antimicrobial sensitivity test

All the 47 isolates that showed red colored ring on indole test were subjected to do antimicrobial sensitivity test to 8 different antimicrobial agents. From the isolates 100% samples are sensitive to Ceftriaxone and Gentamycin which are highest in sensitivity. More than 80% sample shows resistance in Ampicillin, Amoxicillin and Tetracycline. From the isolates, 100% sample shows resistance against Tetracycline.

Table 4: Prevalence of antimicrobial resistance pattern against *E. coli* isolates

Antimicrobial agents	No. of isolates	Sensitivity (%) and zone of inhibition	Intermediately sensitivity and zone of inhibition	Resistance (%) and zone of inhibition
Ceftriaxone	47	47(100) ≥ 18	0	14-17 0 ≤ 13
Gentamycin	47	47(100) ≥ 100	0	13-14 0 ≤ 12
Ampicillin	47	0% ≥ 17	8(17.02)	14-16 39(82.98) ≤ 13
Amoxicillin	47	0% ≥ 18	7(14.89)	14-17 40(85.11) ≤ 13
Ciprofloxacin	47	21(44.68) ≥ 21	14(29.79)	16-20 12(25.53) ≤ 100
Enrofloxacin	47	28(59.58) ≥ 20	12(25.53)	17-19 7(14.89) ≤ 16
Tetracycline	47	0 ≥ 100	0	12-14 47(100) ≤ 11
Colistinsulphate	47	27(57.45) ≥ 14	14(29.79)	11-13 6(12.76) ≤ 10

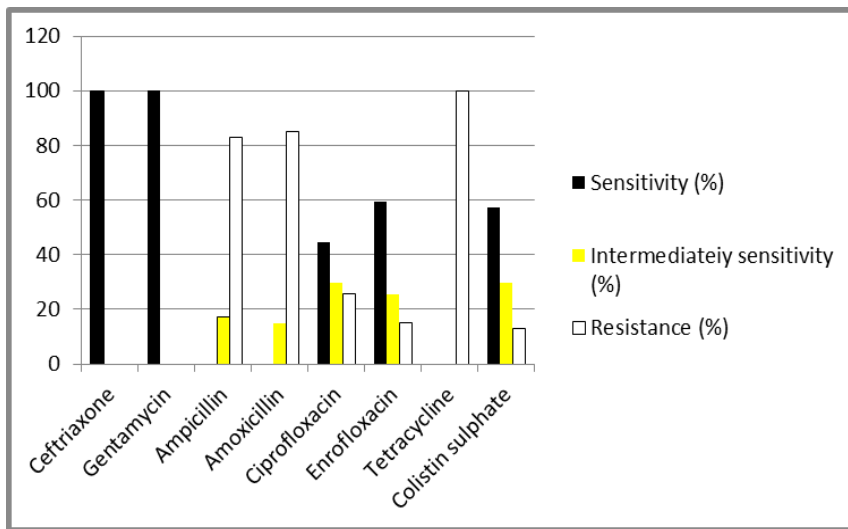


Fig. 1. Graphical representation of prevalence of antimicrobial resistance:

4. Conclusion

From the study it can be concluded that the entire 100 sample collected from liver and yolk sac of DOC investigated for isolation and identification of *E. coli* 47% were positive. From the antimicrobial sensitivity test, it can be said that Gentamycin and Ceftriaxone should be the best drug of choice in case of this day old chick (DOC) of commercial broiler of M M Agha Ltd., Chittagong. Present research findings showed that, the source of single and multiple antimicrobial-resistant of *E. coli* isolates is the frequently used antimicrobials including Tetracycline, Ampicillin, Amoxicillin, Enrofloxacin, Ciprofloxacin. Multidrug resistance of *E. coli* has become a widespread pathogen in Bangladesh. Therefore, it is important to monitor the occurrence of resistance among bacteria from animals and food, as these bacteria are able to spread through food product to human. Finally to determine the prevalence of the various strains, serotypes of *E. coli* and to identify the pattern of antimicrobial resistance, comprehensive study or research should be needed.

5. Discussion

The study was conducted with the aim of isolation and identification of *E. coli* present in liver and yolk sac of DOC. Antibiogram was also done to know the sensitivity and resistance pattern against different antibiotics. Here this study observed that 47% *E. coli* was present in Day Old Chick. The results was partially agreed with the findings of Maiorkaet al. (2006) where he reported that at hatching, chick was still microorganism-free but after hatching a significant number of microorganisms invaded and colonized in different organ. In this study, the average finding of *E. coli* was 47% from DOC of commercial broiler which is similar to the earlier reports of Suhaet al. (2008) who reported 43.50%. Rahman et al. (2004) reported 67.73% and Ahmed et al., (2009) found 52.26% colibacillosis in commercial broiler which is higher than this study. The result is also similar with Bhattacharjee et al., (1996) reported 40.82% of *E. coli* in chicken from Bangladesh. Morphology, staining and cultural characteristics of the bacteria in different cultural media as recorded in the study were almost

similar as reported by Choudhury et al. (1985). They reported that staining and morphology of isolated *E. coli* exhibited Gram negative, small rod, arranged in single or pairs, non-spore former.

Most of the isolates showed resistance against Tetracycline (100%), Amoxicillin (82.98%), and Ampicillin (85.11%). Almost every isolates of this study exhibited multiple resistances to more antibiotics which is similar with few reports from Bangladesh and other parts of the world (Guerra et al., 2003; Khan et al., 2002). Due to abuse antibiotics, such high incidence of multi drug resistance may apparently be occurred (Van de Boogard and Stobberingh, 2000). Most of the isolates showed high sensitivity against Gentamycin (100%) and Ceftriaxone (100%). Present study was agreed with the previous study, where the poultry *E. coli* isolates were found resistant to tetracycline (Biswas et al., 2001). We suspected high level of resistance against Tetracycline because most of the farmers used commercially available tetracycline in the poultry feed regularly. The farmers used antibiotics largely for three purposes in poultry farms: therapeutic use to treat sick flock; prophylactic use to prevent infections in the flock; as growth promoters to improve feed utilization and production (Barton, 2000). Besides, isolates were sensitive 100% to Gentamycin. On the other hand, most of the environmental strains were 97% sensitive to Gentamicin (Alamet al., 2006). Tricia et al., 2006 reported 43% isolates of *E. coli* were resistant to ampicillin but no isolate was found resistant to gentamicin. So, the results of Gentamycin sensitivity in poultry isolates were mostly similar with Environmental *E. coli*.

Conflicts of interest

The authors declare no conflicts of interest.

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