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

Muhammad Ali Akond 1,2*, Saidul Alam 1, S.M.R. Hassan 1, Momena Shirin 3

1Department of Botany, Jahangirnagar University, Dhaka-1342, Bangladesh
2Lab of Environmental Bioscience, Faculty of Agriculture, Yamaguchi University, Japan 753-8515
3Institute of Public Health, Mohakhali, Dhaka 1212, Bangladesh.

**Abstract:** Isolation and identification of *Escherichia coli* were made from poultry sources of different poultry markets in the capital city of Bangladesh. Out of total 250 samples, 50 from each of cloacal swab, intestinal fluid, egg surface, faecal material and hand wash of chicken handlers, 145 (58%) were found to be positive for *E. coli* prevalence. 80 selected strains were thoroughly characterized by standard cultural and biochemical tests followed by final identification using latex agglutination test with several polyvalent anti-sera. 50 identified strains were subjected to 13 antimicrobial agents to check their susceptibility. 88%, 82%, 80%, 76%, 70%, 68%, 64%, 58%, 52%, and 20% of the tested *Escherichia coli* strains from poultry sources were found resistant respectively to Penicillin, Ciprofloxacin, Riphampicin, Kanamycin, Streptomycin, Cefixine, Erythromycin, Ampicillin, Tetracycline, and Chloramphenicol and Neomycin. None of the strains showed resistance to Norfloxacin and Gentamicin. Sensitivity was recorded in case of 86%, 80%, 60%, 36%, 30%, and 26% of the strains to Norfloxacin, Gentamicin and Chloramphenicol, Neomycin, Tetracycline, Streptomycin and Ampicillin, respectively. 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 recorded in case of 6-10 antibiotics for all strains tested. More cautions are recommended for personnel hygiene in processing and handling of poultry and poultry products. Excess use or abuse of antibiotics should be reduced or stopped by judicious application of antibiotics for the safety of public health.

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

*Corresponding author: Lab of Environmental Bioscience, Faculty of Agriculture, Yamaguchi University, Japan 753-8515. Tel: +81-80-3056-7275, E-mail: akond316@yahoo.com

**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 (Jawetz et al. 1984; Levine 1987). Although most isolates of *E. coli* are nonpathogenic but they are considered as indicator of faecal contamination in food and about 10 to 15% of intestinal coliforms are opportunistic and pathogenic serotypes (Barnes and Gross 1997) 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 (Daini et al. 2005) and yolk sac infection, omphalitis, cellulitis, swolled head syndrome, coligranuloma, and colibacillosis (Gross 1994). During the past two decades, severe outbreaks of gastrointestinal illness have occurred by food borne pathogenic *E. coli*, especially 0157:H7 (Armstrong et al. 1996).

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 (Moreno et al. 2000; Okeke et al. 1999). Moreover, antibiotic treatment is considered the most important issue that promotes the emergence, selection and spreading of antibiotic-resistant microorganisms in both veterinary and human medicine (Neu 1992; Witte 1998). 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 (Van de Bogaard et al. 2001; Schroeder et al. 2002). 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* (Bensink and Botham 1983; Lakhotia and Stephens 1973; Turtura et al. 1990).
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 of Dhaka, Bangladesh.

**Sampling from cloacal swab.** Sterile swab stick moistened with sterile normal saline water was inserted in the cloaca 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°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 hours at 37°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 (1974) 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 (Bauer et al. 1966) 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 minutes 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 to 5 minutes. 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 minutes of the
application of the discs, the plates were inverted and incubated at 37°C. After 16 to 18 hours 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 and Discussion

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 Table 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 surface to 82% in feces.

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

| Sample Source         | No. of Samples Tested | No. of Samples Positive for E. coli Detection | Percentage positive samples      |
|-----------------------|-----------------------|-----------------------------------------------|----------------------------------|
| Cloacal 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                               |

The prevalence of E. coli in faecal samples (82%) in this study was higher than the previous records of Nazir (2004) and Rahman et al. (2008). The egg surface was contaminated with E. coli probably from poultry feeds and/or feces during lay in unhygienic condition or also from infected poultry. Among the animal protein ingredients, a major ingredient of poultry feeds, locally processed cheap fish wastes were found to be important causes for bacterial contamination of poultry feeds (Ekwuagana 2004). E. coli was reported as a common microflora in raw feeding materials and poultry feeds (Da Costa et al. 2007). 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.

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, Rhipampicin, Kanamycin, Streptomycin, Cefixime, Erythromycin, Ampicillin, Tetracycline, and Chloramphenicol and Neomycin (Table 3). No strain was found either sensitive to erythromycin, rhipampicin,

Table 2: Biochemical tests used for identification of Escherichia coli

| Biochemical Test Properties | 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                                        |
| Methyl Red Test             | +                         | 75                                        |
| Voges-Proskauer Test        | +                         | 60                                        |
| 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.

kanamycin, cefixime, 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.

Resistance of E. coli isolates from Malaysian broiler chicken to ampicillin, tetracycline and gentamicin with 11-95% range has been reported (Apun et al. 2008). Rahman et al. (2008) 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 (Islam et al. 2008). Tricia et al. (2006) 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 (2008) 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 (Guerra et al. 2003; Khan et al. 2002;
Table 3: Antibiotic susceptibility pattern of 50 selected strains of *Escherichia coli*

| Antibiotic Discs | 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 |
| Rhipampicin (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 |
| Cefixime (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 | 86.0 | >17 |
| Ciprofloxacin (5 µg) | 82.00 | <30 | 18.00 | 30-33 | -- | >33 |

Rahman et al. 2008; Zhao et al. 2005). 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 (Van de Boogard and Stobberingh 2000).

Reduction in the frequency of vancomycin resistant *Enterococci* from broilers from 80% to 5% due to ban imposed on avoparcin as a feed additive for poultry in Denmark 1995 (Aarestrup et al. 1998) suggests to encounter this resistance emergence with reduced and judicious application of antibiotics in animal farming and clinical purposes.

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References

Aarestrup FM, Bager F, Madsen M, Jensen NE, Meyling A, Egener HC. 1998. Surveillance of antimicrobial resistance in bacteria isolated from food animals to antimicrobial growth promoters and related therapeutic agents in Denmark. APMIS. 106: 106.

Amara A, Ziani Z, Bouzoubaa K. 1995. Antibioreistance of *Escherichia coli* strains isolated in Morocco from chickens with colibacillosis. Vet. Microbiol. 43: 325-330.

Apun K, Chong YL, Abdullah MT, Micky V. 2008. Antimicrobial Susceptibilities of *Escherichia coli* Isolates from Food Animals and Wildlife Animals in Sarawak, East Malaysia. Asian Journal of Animal and Veterinary Advances 3(6): 409-416.

Armstrong GL, Hollingsworth J, Morris JG. 1996. Emerging food borne pathogens: *Escherichia coli* 0157: H7 as a model of entry of a new pathogen into the food supply of the developed world. Epidemiological Review 18: 29-51.

Barnes HJ and Gross WB. 1997. Colibacillosis. Pages 131–139 in Diseases of Poultry. 10th ed. B. W. Calnek, ed. Mosby-Wolf Publication Ltd., London, UK.

Bauer AW, Kirby WM, Sherris JC, Turck M. 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45(4): 493–496.

Bensink JC, Botham FP. 1983. Antibiotic resistant coliform bacilli, isolated from freshly slaughtered poultry and from chilled poultry at retail outlets. Aust. Vet. J. 60: 80–3.

Buchanan RE, Gibbons NE (eds.). 1974. Bergey’s manual of determinative Bacteriology. 8th ed. Baltimore: The Williams and Wilkins.

Da Costa PM, Oliveira M, Bica A, Vaz Pires R, Bernardo F. 2007. Antimicrobial resistance in *Enterococcus* spp. and *Escherichia coli* isolated from poultry feed and feed ingredients. Vet. Microbiol. 120: 122-131.

Daini OA, Adesemowo A. 2008. Antimicrobial Susceptibility Pattern and R- Plasmids of Clinical Strains of *Escherichia coli*. Aus. J. Basic and Applied Sci. 2(3): 397-400.

Daini OA, Ogbulu OD, Ogunleedun A. 2005. Quinolones Resistance and R-plasmids of some gram negative enteric Bacilli. Afr. J. Clin. and Exp. Micro. 6: 14-19.

Davies J. 1994. Inactivation of antibiotics and the dissemination of resistance genes. Sci. 264: 375-382.

Ekwuagana IC. 2004. Frequency of isolation of *Salmonella* from feed raw materials and their antimicrobial resistance profile. B. Agric. Tech. Project Report, Federal University of Technology, Owerri, Nigeria.
Gross WB. 1994. Diseases due to Escherichia coli in poultry. pp. 237–260. In Gyles CL (ed.) Escherichia coli in domesticated animals and humans. CAB International. Wallingford, UK.

Guerra B, Junker E, Schroeter A, Malorny B, Lehmann S, Helmhut R. 2003. Phenotypic and genotypic characterization of antimicrobial resistance in German Escherichia coli isolates from cattle, swine and poultry. J. Antimicrob. Chemother.52 (3): 489-92.

Gupta K, Hooton TM, Stamm WE. 2001. Increasing antimicrobial resistance and the management of uncomplicated community-acquired urinary tract infections. Ann. Int. Med. 135: 41–50.

Islam MJ, Sultana S, Das KK, Sharmin N, Hassan MN. 2008. Isolation of plasmid mediated multidrug resistant E. coli from poultry. Int. J. Sustain. Crop Prod. 3(5): 46-50.

Jawetz E, Melnick J, Adelberg EA. 1984. Review of Medical Microbiology. 16th ed. Los Altos, California: Long Medical Publication, pp. 122-144.

Khan A, Das SC, Ramamurthy T, Sikander A. 2002. Antibiotic Resistance, Virulence Gene, and Molecular profile of Shiga Toxin-Producing Escherichia coli Isolates from Diverse Source in Calcutta, India. J. Clin. Microbiol. 40(6): 2009-2015.

Lakhotia RL, Stephens JF. 1973. Drug resistance and R factors among enterobacteria isolated from eggs. Poult. Sci.52: 1955-1962.

Levine M. 1987. Escherichia coli that cause diarrhea: enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic and enteroadherent. J. Infect. Dis.155: 377-390.

Marshall B, Petrowski D, Levy LV. 1990. Inter- and intraspecies spread of Escherichia coli in a farm environmental in the absence of antibiotic usage. Microbiol. 87: 6609-6613.

Moreno MA, Dominguez L, Teshoger T, Herrero IA, Perrere ME. 2000. Antibiotic resistances monitoring: The Spanish Programme. Int. J. Antimicrob. Agents.14: 285-290.

Nazir KHMNH. 2004. Molecular base of diversified E.coli isolates potentiating antibiotics resistant and comprising epidemiology. M.S. Thesis, Bangladesh Agriculture University. Mymensingh, Bangladesh.

Neu, H. C. 1992. The crisis in antibiotic resistance. Science 257: 1064–73.

Okeke IN, Lamikanra A, Edelman R. 1999. Socio-economic and behavioral factors leading to acquired bacterial resistance to antibiotics in developing countries. Emerg. Infect. Dis.5: 13-27.

Osterblad M, Hakanen A, Manninen R, Leistevuo T, Peltonen R, Meurman O, Huovinen P, Kotilainen P. 2000. A between- species comparison of antimicrobial resistance in enterobacteria in fecal flora. Antimicrob. Agents Chemother. 44: 1479-1484.

Rahman M, Rahman BM, Rahman B. 2008. Antibiogram and plasmid profile analysis of isolated Escherichia coli from broiler and layer. Res. J. Microbiol. 3 (2): 82-90.

Schroeder CM, Zhao C, Debroy C, Torcolini J, Zhao J, White DG. 2002. Antimicrobial resistance of Escherichia coli 0157:H7 isolated from humans, cattle, swine and food. Applied Environ. Microbio. 68: 576-581.

Tricia DM, McLaughlin W, Brown PD. 2006. Antimicrobial resistance of Escherichia coli isolates from broiler chickens and humans. BMC. Vet. Res. 2: 7.

Turtura GC, Massa S, Chazvinizadeh H. 1990. Antibiotic resistance among coliform bacteria isolated from carcasses of commercially slaughtered chickens. Int. J. of Food Microbiol.11: 351–4.

Van de Boogard AE, Stobberingh EE. 2000. Epidemiology of resistance to antibiotics links between animals and humans. Int. J. Antimicrob. Agents. 14: 327-335.

Witte W. 1998. Medical consequences of antibiotic use in agriculture. Science. 279: 996–97.

Zhao S, Maurer JJ, Hubert S, De Villena JF, McDermott PF, Meng J, Ayers S, English L, White DG. 2005. Antimicrobial susceptibility and molecular characterization of avian pathogenic Escherichia coli isolates. Vet. Microbiol. 107 (3-4): 215-24.