

## ISOLATION OF PLASMID- MEDIATED MULTIDRUG RESISTANT *Escherichia coli* FROM POULTRY

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### ABSTRACT

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The study was conducted in Chittagong University, Bangladesh during January 2007 to April 2007 to investigate the relationship between the use of antibiotic and the development of resistance, 30 strains of *Escherichia coli* were isolated from fecal waste of chickens from Denm poultry farm, Chittagong, Bangladehs. *E. coli* strains were analyzed by agar disc diffusion to determine their susceptibility patterns to 7 antimicrobial agents. Plasmid DNA was isolated from ten isolates taken randomly by agarose-gel electrophoresis. Results of antibiotic susceptibility test showed that all of the isolates are multi-drug resistant ( $\geq 4$ ) and were resistant to Tetracycline (96.6%), Ciprofloxacin (30%), Penicillin (100%), Erythromycin (66.6%), Gentamycin (50%) and Chloramphenicol (100%) but all of them were sensitive to Imipenem. Turbidometric analysis of ciprofloxacin resistance pattern showed all of the isolates are highly resistant to ciprofloxacin even at concentration of 3000 $\mu$ g/ml. Agarose-gel electrophoresis of plasmid DNA from 10 isolates showed that all of them contain a high-molecular weight plasmid DNA. Our study highlight that the resistance development is directly related to use of antibiotics. the results suggest that the multi-drug resistant *E.coli* & plasmid containing multidrug resistant genes are present in the hospital waste may act as a possible source of transfer of these highly resistant pathogens and their genes to human that could be threat for the treatment of disease by commercially available antibiotics.

**Keywords:** Multidrug resistant, *Escherichia coli*, poultry, plasmid

### INTRODUCTION

About 65 years ago, from the time when antibiotics became widely available, they have been acclaimed as miracle drugs talented to destroy disease-causing bacteria. But with each transitory decade, bacteria that resist not only single, but multiple, antibiotics making some diseases particularly troublesome to control have become progressively more prevalent. Antimicrobial resistance take place when bacteria adjust or adapt in a way that permits them to stay alive in the presence of antibiotics designed to kill them. , bacteria evolve resistance to these drugs, typically by acquiring chromosomal mutations and multidrug resistant plasmid & transposon etc (Finch *et al.*, 2003; Lautenbach *et al.*, 1987; Levine *et al.*, 2002; Nichol *et al.*, 2003; Pena, 1995; Sheng, 2002).The worldwide emergence of antibiotic-resistant bacteria threatens to undo the dramatic advances in human health that were ushered in with the discovery of these drugs in the mid-1900s. Today, resistance has rendered most of the original antibiotics obsolete for many infections, mandating an increased reliance on synthetic drugs (Cirz *et al.*, 2003). In poultry production antibiotics are widely used as growth promoter and treatment of infectious disease (Wolfgang, 1998). The use of antibiotics in the poultry production industries for the promotion of growth largely contributes to the high resistance to antimicrobial agents in normal flora of poultry (Allan *et al.*, 1993; Aronson *et al.*, 1975) and pathogenic microorganisms (Amara, *et al.*, 1995). These resistance microorganisms may act as a possible source for the transfer of antimicrobial resistance to human pathogens (Van den *et al.*, 2001). Plasmid and transposon-mediated resistance is widely transmitted between different bacterial species and genera including human pathogens (Davies, 1994; Wise, 1985). Multi-drug resistant strains of *E.coli* are prevalent in both human and animal isolates in different parts of the world (Amara *et al.*, 1995; Bebora *et al.*, 1994; Mahipal *et al.*, 1992). *E.coli* is a common normal flora organism in the gastrointestinal tract of animals and man but may become pathogenic to both (Jacobs *et al.* 1970; Levine *et al.* 1987). Serious outbreak of gastrointestinal illness caused by foodborne pathogenic *E.coli*, have occurred during the past two decades (Armstong *et al.*, 1996). Thus resistant strain of *E.coli* arising from the exposure of animals to antimicrobials may possibly become infectious organisms in humans. Antimicrobial agents are widely used in poultry industry include B-lactamase, tetracycline, aminoglycosides, macrolides, fluoroquinolones etc. our aims to correlate the use of antibiotics in the poultry industries and the resistance development.

### MATERIAL AND METHODS

#### *Isolation and Identification of Tetracycline resistant Escherichia coli*

With the aim of investigation of antibiotics resistant bacteria and to establish the correlation of the use of antibiotic in the poultry for growth promotion and treatment & control of disease and the resistance development, we collect

the fecal sample from the different places of some poultry farms in Hathazari, Chittagong. Sample were diluted and directly inoculated into MacConkey agar, Eosine Methylene blue, Xylose-lysine deoxycholate agar plate containing 30µg/ml tetracycline. Subsequently a total of 30 isolates were then identified by standard laboratory methods including Gram staining, IMViC test.

#### ***Antimicrobial susceptibility analysis***

The Bauer Kirby disc diffusion method (Bauer AW. et al. 1999) was used to test susceptibility of the isolated and identified organisms to Penicillin (30µg/ml), Tetracycline (30µg/ml), Erythromycin (15µg/ml), Chloramphenicol (30µg/ml), Gentamycin (10µg/ml), Imipenem (10µg/ml), and Ciprofloxacin (5µg/ml). All discs were obtained from Oxoid (Unipath Ltd, Basings take, UK). Interpretation of the followed criteria recommended in the National Committee for clinical Laboratory Standards (NCCLS. 1993). By using light absorbance technique, the growth of bacteria in broth was measured at 590nm in a spectrophotometer. Then the resistant pattern was analyzed by drawing a graph using the chart of absorbance in different concentrations of tetracycline (40µg/ml to 150µg/ml) in case of samples collected from hospital waste. Treatment history collected from the poultry farm during the sample collection time March 2007 to April 2007. The treatment history is important to predict the correlations between the antibiotic use in the poultry farms and the development of antibiotic resistance.

#### ***Plasmid isolation***

Plasmid extraction was carried out by alkaline lysis technique (Bonfiglio G. et al. 1995). The extracted plasmid was then isolated using a horizontal 1% agarose gel electrophoresis technique.

#### ***Preparation of the cells***

The pure cultures were transferred to 10 ml screw cap tubes containing 3 ml Luria Bertani (LB) broth with 30 µg/ml ciprofloxacin. The broths were then incubated at 37°C with loose capping and vigorous shaking (>250 rpm) for overnight. Then inoculums were transferred to another 3 ml LB broth at a 1:200 ml rate containing same concentration of ciprofloxacin and incubated for 4-6 hours at 37°C with loose capping and vigorous shaking (>250 rpm). After a sufficient growth with slight turbidity the incubation stopped and the cells were prepared for extraction.

#### ***Plasmid extraction procedure***

1.0 ml of overnight culture was taken in an Eppendorf's tube (1.5ml) and cells were collected by centrifugation for 7 minutes at 12,000 rpm. The supernatant was discarded. The pellet was thoroughly suspended in 100 µl of solution I and the solution was kept at room temperature (32°C) for 10 min. Then 200 µl of solution II (lysis solution) was added and mixed gently by inverting the tube for a few times. After that 150 µl of ice-cold solution III (neutralizing solution) was added and mixed vigorously by vortexing for a few seconds. The tubes were kept on ice for 5 min. The mixture was then centrifuged at 12,000 rpm for 15 minutes to pellet the chromosomal DNA. The clear supernatant was (approximately 400 µl) was taken to fresh Eppendorf's tubes. Then two volumes of cold 95% ethanol (800 µl) was added in each tube and vortexed for a few seconds to mix well. It was then kept in room temperature for about 20 min for DNA precipitation. The precipitated DNA was collected by centrifugation for 15 minutes at 12,000 rpm. The supernatant was discarded. The pellet was dried in a drier at 45°C for 20 minutes. At last the dried DNA was dissolved in 30 µl TE buffer and kept at 4°C.

#### ***Separation of plasmid DNA by agarose gel electrophoresis***

Plasmid DNA was separated by horizontal electrophoresis in 1% agarose slab gels in a Tris-Acetate EDTA (TAE) buffer at room temperature at 80 volt (50 mA) for 4 h. Briefly, 30 µl of plasmid DNA solution was mixed with 3 µl of tracking dye and was loaded into the individual well of the gel. The gel (5mm thick) was stained with 0.5 µg/ml of ethidium bromide for 15 min at room temperature and then destained with distilled water for 10 min. DNA bands were visualized and photograph was taken using the apparatus Bio-rad, Wide Mini-Sub Gel GT. The molecular weight of the unknown plasmid DNA was determined on the basis of its mobility through agarose gel. Plasmids were present in *E. coli* isolates.

## **RESULTS AND DISCUSSIONS**

Analysis of treatment history collected from the poultry farm showed that they used mainly tetracycline (96.6%) for the growth promotion purposes and quinolones (20%), Macrolides (20%) for the treatment purposes. Results of antibiotic susceptibility test showed that all of the isolates are multi-drug resistant ( $\geq 4$ ). All of the isolate were

resistant to tetracycline (96.6%), ciprofloxacin (30%), penicillin (100%), erythromycin (66.6%), Gentamycin (53.3%) and chloramphenicol (100%). Turbidometric analysis of tetracycline resistance pattern showed all of the isolates are resistant even at concentration of 150µg/ml. Agarose-gel electrophoresis of plasmid DNA from 10 isolates showed that all of them contain a high-molecular weight plasmid DNA.

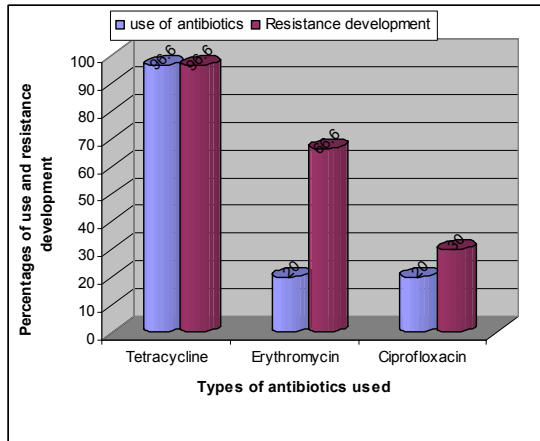


Figure 1. Comparative analysis of antibiotics use in poultry and the resistance development

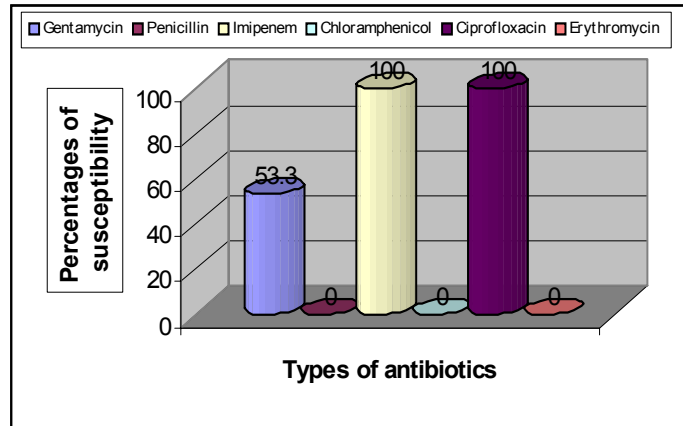


Figure 2. Susceptibility of tetracycline-resistant isolate to Other antibiotics

We investigated the resistance pattern of 30 isolates of *Escherichia* isolated from poultry of seven antimicrobials of which 3 were commonly used in the poultry farms clearly demonstrated the high resistance rate to all of these three tested antibiotics commonly used in the poultry farms but not to those which are used for the treatment purposes (Mastour S. et al. 1999). This result suggest that the extent of resistance is to an antibiotic is associated with the extent of antibiotic use (Bebora LC et al.1997; Mastour S. et al. 1999). *E. coli* isolated for the poultry farm was highly resistant to tetracycline even at 150µg/ml which could be the result of misuse and repeated use of tetracycline for the promotion of growth in the poultry farm (Allen DG et al. 1993). High resistance rate was also noted against chloramphenicol and penicillin could be associated with the misuse of them as reported by Ginns *et al* (1999). All of the isolates found multiple antibiotic resistance (>=4) as reported by Zhao *et al.* (2005); Guerra *et al.* (2003); Khan *et al.* (2002);Mulamattathil S.G *et al.* (2000); Kariuki *et al* (1999).

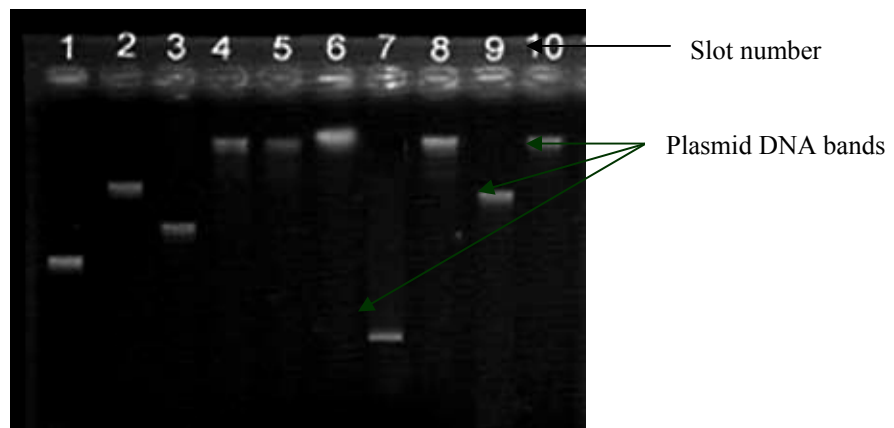


Figure 3. Plasmid profile of multidrug resistant *Escherichia coli* strains

We analyzed randomly 10 isolates for the presence of Plasmid DNA and 7 of them found to contain high molecular weight plasmid with high resistant rate as reported by John *et al* (2002). Our study conclude that multiple resistant *E.coli* isolates and plasmid containing multidrug resistant genes are present in the poultry farms and the poultry may act as a possible source of transfer of these highly resistant pathogens and their genes to human as reported by Wooley *et al* (2007), Mastour *et al.* (1999), we concur with the calls to ban the use off antibiotics for growth promotion and treatment in the poultry sectors.

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