INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com Volume-4, Issue-1, Jan-Mar-2013 Coden : IJABPT Copyrights@2013

ISSN : 0976-4550

*Received: 07th July-2012* *Revised: 10th July-2012* *Accepted: 13th July-2012*

***Research article***

# RESISTANCE PATTERN OF FECAL *ESCHERICHIA COLI* IN SELECTED BROILER FARMS OF EASTERN HARARGHE ZONE, ETHIOPIA.

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**ABSTRACT** : A study was conducted to determine the pattern of antimicrobial resistance in Escherichia coli isolated from Cloacal swab of broiler chickens in selected farms of Eastern Harrarge zone of Ethiopia. Isolation and identification of Escherichia coli were done by using enrichment media, selective media, and biochemical tests. 65 selected isolates were subjected to 9 antimicrobial agents to determine their resistance by the disk diffusion method. Accordingly, the resistance of E.coli was tetracycline (90%), streptomycin (78%), ampicillin (60%), amoxicillin (56%), erythromycin (45%), ciprofloxacin (38%), and chloramphenicol (15%). None of the isolates showed resistance to gentamicin. Sensitivity was observed in case of 80%, 77%, 44%, 32%, 26%, 20%, 20%, 15%, and 10% of the isolates for chloramphenicol, gentamicin, ciprofloxacin, amoxicillin, ampicillin, streptomycin, erythromycin, and tetracycline, respectively. Intermediate resistance/susceptibility was recorded for 5-35% of the isolates. 92.3% of the isolates tested showed multidrug resistance for 2 or more antimicrobials and the highest levels (18.5%) of multidrug-resistant E. coli were observed for 3 antimicrobials accounting 7.7% for tetracycline-ampicillin-streptomycin and 10.8% for tetracycline-ampicillin-amoxicillin. This study showed resistance against the antibiotics that are commonly used in poultry. Furthermore, it was concluded that gentamicin, chloramphenicole and ciproflaxin will be the first drugs of choice to resist infections caused by E. coli in chicken in Ethiopia. These findings confirm significant increase in the incidence of antimicrobial resistance in the E. coli isolates which is most probably due to increased use of antibiotics as feed additives for growth promotion and prevention of diseases and use of inappropriate antibiotics for treatment of diseases. Hence, excess or abusive use of antimicrobials should be guarded through judicious application of antimicrobials.

**Key words:** *Antimicrobial resistance, Escherichia coli, Poultry farm, Ethiopia.*

## INTRODUCTION

Antimicrobials are an integral part of animal farming to treat clinical diseases and to increase productivity through the use of these agents as essential feed additives to maintain healthy flock (Witte, 1998). Antibiotic usage remains one of the most important factors that promote the emergence, selection and dissemination of antibiotic resistant microorganisms in both veterinary and human medicine (Witte, 1998). Use of antimicrobials for therapeutic purpose has resulted in antimicrobial resistance and consequently, loss of efficacy of this antimicrobial agent (Miranda et al., 2008; Phillips et al., 2003). It's very common practice in intensive poultry farming to administer antibiotics to whole flocks rather than individual animals through feed or drinking at a sub therapeutic dose for use as a prophylaxis, metaphylaxis and growth promotion purposes which could potential lead to antibiotic resistance (Hart et al., 2004;, Moreno et al., 2000).

When antibiotic were firsts used in chemotherapy, development of antibiotic-resistant microorganism was infrequent. However, with the very wide use of antibiotics, resistance becomes much more of problem as susceptible microbes were eliminated and numbers of resistant microrganisms increased [6]. This selection pressure for resistance in bacteria in poultry consequently leads to harboring of fecal flora that contain a relatively high proportion of resistant bacteria (Van Den Bogaard and Stobberingh, 1999). *Escherichia coli (E. coli*) are one of the most important bacteria against which many antimicrobials have been tried and a variable degree of resistance has been recorded in both humans and animals (Amara et al., 1995). This bacterium is known to acquire resistance faster than any other bacteria. Changes in resistance of this bacterium have been used as an indicator of resistance in potentially pathogen bacteria (Von Baum and Marre, 2005). Included in the R-factor plasmids, which is common in the enterobacteria, is the resistance transfer factor and genes for resistance to several antibiotics. The development of resistance in bacteria to antibiotic usually involves a stable genetic change, heritable from generation to generation. Many mechanisms that result in the alteration of bacterial genetic composition can influence the emergence of resistance.

Resistance to multiple antimicrobial agents quite commonly creates an extensive problem in treating both intestinal and extraintestinal infections of *E. coli* (Gupta et al., 2001). Moreover, resistant *E. coli* may also function as a potential source in the transportation of antimicrobial resistance to human pathogens. Antibiotic sensitivity testing should be carried out with any isolate that is considered to be significant, before the treatment of animal with antimicrobial drugs. Studies focusing on antibiotic resistance should be practice in order to avoid the evolving antibiotic resistance *E. coli* which might seriously affect productivity and elimination of poultry farms (Ronald, 1997). Few or no studies have been conducted in Ethiopia to determine the antibiotic resistance of *E. coli* isolates in poultry. Therefore, the purpose of this study was to isolate fecal *E. coli* from selected broiler farms in Eastern harrarge zone of Ethiopia and determine the resistance patterns to some selected antimicrobials.

## MATERIALS AND METHODS

### Sampling Site

A total of 150 fecal samples were collected from Cloacal swabs of broiler chickens from different intensive poultry farms in eastern Harrarge Zone of Ethiopia.

### Sample Size Determination

In order to detect at least one animal with *E. coli* on a farm, the general formula used by Smith (1995) was used to compute sample size (*n*inf): $n_{inf}$= log(α)/log(1 - prev), where α is the probability that none of the sampled animals harbor *E. coli* and prev is expected prevalence of *E. coli*. We assumed that the expected prevalence of *E. coli* was 10% and that the type I error was 0.05. Using the equation and assumptions described above, we calculated that 29 animals was the minimum number necessary for testing.

### Sample Collection

About 50 gm of fresh fecal samples was collected aseptically from poultry cases into sterile vials using a sterile swab stick which was moistened with sterile normal saline water inserted in the cloacae of chickens and the samples collected were immediately transferred into a sterile vial containing 10ml of buffer peptone water (BPW), which was used to avoid drying out of the swabs. All the samples collected were transported in an ice box with ice packs to the Veterinary Microbiology Laboratory at the College Veterinary Medicine. Samples were then incubated in buffered peptone water (BPW; Oxoid, Hampshire, England) at a ratio of 1:10.

### Isolation and Identification of Escherichia coli

Isolation and identification of *E. coli* were performed by standard bacteriological methods (Quinn et al., 2002). Briefly, a $10^{-3}$ dilution of each specimen was made in peptone water and 50 µl were placed on MacConkey agar and incubated at $37^0$C for 24h. The next day the colonies appeared pink considered produce presumptive of *E. coli* colonies and were sub cultured on to nutrient agar (non-selective media) to get a pure culture. Gram's staining revealed gram's negative small rods. Colonies from each plate were then picked up and streaked on to an eosin-methyline-blue (EMB) agar plate, which produced a typical metallic sheen. Presumptive *E. coli* colonies were then confirmed by panels of biochemical tests (i.e. Oxidase, Catalese Indole, Methyl red, Voges-Proskauer reaction and Simmons citrate (IMViC), urea hydrolysis, gelatin hydrolysis, lactose fermentation, nitrate reduction, casein hydrolysis and sugar fermentation tests).

### Antimicrobial Susceptibility Test

Antimicrobial susceptibility test of each of the isolates was determined by the Kirby-Bauser disc diffusion method (Bauser et al., 1966) as per the recommendation of National Committee for Clinical Laboratory Standards (NCCLS, 1997). This method allowed for rapid determination of in-vitro efficacy of a drug by measuring the diameter of the zone of inhibition that results from diffusion of the agent into the medium surrounding the disc. The *E. coli* isolates were tested against the antibiotics of human and veterinary significance. A total of 9 antimicrobials discs (Becton Dickinson, U.S.A.) impregnated with, Streptomycin (S) 10µg, Chloramphenicol (CAF) 30µg, Ciprofloxacin (CIP) 5µg, Tetracycline (TTC) 30µg, Erythromycin (EM) 15µg, Ampicillin (AMP) 30µg, Amoxicillin (AMO), Sulfomethoxazole-thrimethroprim (SXT) 25µg and Gentamycin 30µg were applied on Mueller Hinton agar using a sterile forceps. Five discs (four antibiotics discs and one blank disc as control) were placed in each petri dish. The plates were then inverted and incubated at 37°c for 24 hours. The next day the plates were examined and the zones of inhibition around each antimicrobial were measured using calipers to the nearest millimeter and interpreted as susceptible, intermediate or resistant according to the interpretation table of the Becton Dickinson Microbiology Company, USA.

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## RESULTS

In the present study 65 *Escherichia coli (E. coli*) isolates were recovered from the Cloacal samples. All these isolates were subjected to the antibiotic susceptibility test. Antibiotic resistance pattern of the E. coli isolates has been presented as susceptible, intermediate and resistant in figure 1. The study revealed that all fecal *E. coli* isolates from broilers showed a variable degree of prevalence of resistant to 8 of the 9 antimicrobial tested. The resistance prevalence in a decreasing order was 90%, 78%, 65%, 60%, 56%, 45%, 38%, 15.0%, and 0.0%, for tetracycline (TTC), sulfomethoxazole-thrimethroprim (SXT), streptomycin (S), ampicillin (A), amoxicillin (AMO), erythromycin (E), ciprofloxacin (CIP), chloramphenicol (CAF), and gentamicin (GM), respectively. Moreover, 5.0-35.0% of isolates was found intermediate resistant to 8 of the 9 antibiotics tested. Likewise, the antibiotic prevalence for susceptibility ranges 10.0-77.0% for the 9 antibiotics tested. None of the isolates tested showed resistance to GM. In the present study almost all isolates showed single resistance to 9 to 10 antibiotics tested.

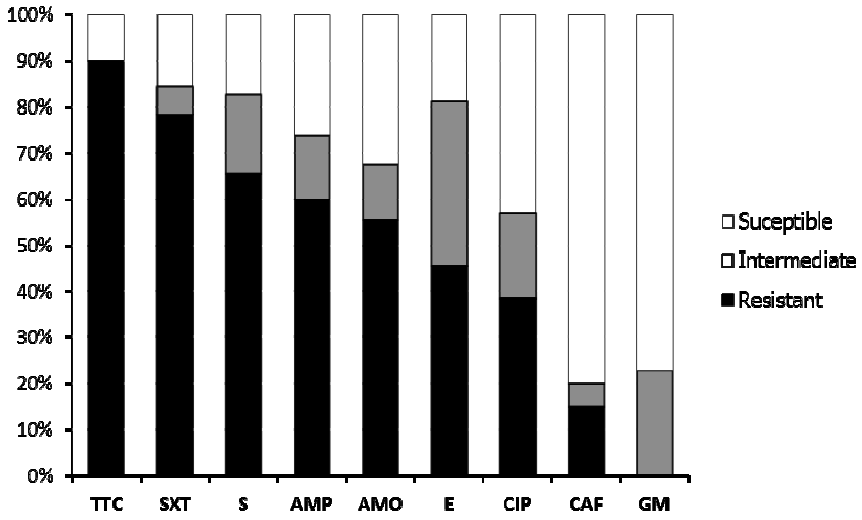


**Figure. 1 Antibiotic resistance patter of *E.coli* isolates to TTC: tetracycline, SXT: sulfomethoxazole-thrimethroprim, S: streptomycin, AMP: ampicillin, AMO: amoxicillin, E: erythromycin, CIP: ciprofloxacin, CAF: chloramphenicol, GM: gentamicin.**

The present study revealed the highest percentages of resistant *E. coli* isolate (96.9%), out of which 92.3% of them are multi-drug resistant isolates to at least 2 antibiotics. Moreover, about 12.3% of the isolates were resistant to 5 or 6 antibiotics. Only two isolates showed no resistance to all the antimicrobials tested. The percentages of prevalence of multi-drug resistant patterns in the fecal isolates of *E. coli* are outlined in Table 2.

**Table 1: Prevalence (%) of multiresistant *E.coli*.**

| Number of Antibiotics | No (%) of isolates resistant to the 65 isolates tested |
|---|---|
| 0 | 2 (3.1) |
| 1 | 3(4.6) |
| 2 | 13(20.0) |
| 3 | 29(44.6) |
| 4 | 10(15.4) |
| >=5 | 8(12.3) |

Out of the 92.3% of multi-resistant isolates, the highest level of resistance (18.5%) observed for 3 antibiotics accounting 7.7% for tetracycline-ampicillin-streptomycin and 10.8% for tetracycline-ampicillin-amoxicillin. In of the most frequent multi-resistant patterns, TTC was recorded as the common antibiotic against which *E.coli* was resistant (Table 3).

**Table 2: The most frequent multidrug resistance pattern of fecal *E.coli***

| Antibiotics pattern* | Percentage of isolates |
|---|---|
| TTC-AMO | 5.4 |
| TTC -AMP | 3.6 |
| TTC-AMP-S | 8.9 |
| TTC-AMP-AMO | 12.5 |
| TTC-AMO-CAF | 3.6 |
| TTC-AMO-SXT | 5.4 |
| TTC-AMO-AMP-SXT | 3.6 |
| TTC-AMP-S-SXT-CAF | 1.8 |

* TTC: tetracycline, SXT: sulfomethoxazole-thrimethroprim, S: streptomycin, AMP: ampicillin, AMO: amoxicillin (AMO), CAF: chloramphenicol.

## DISCUSSION

The antimicrobial susceptibility data from the present study showed that poultry fecal material in Ethiopia harbor *E. coli* resistant to various antimicrobials commonly used in veterinary and human medicine. It was observed that very high percentage of *E. coli* isolates were sensitive to chloramphenicole (80%), trimethoprim-sulfamethoxazole (78%), Gentamycin (77%), and ciproflacin (44%) but resistant to other drugs such as tetracycline (90%), trimethoprim-sulfamethoxazole (78%), streptomycin (65%), ampicillin (60%), amoxicillin (56%) and erythromycin (45%).

In this study a higher resistance rate against the antibiotics that are commonly used in poultry it was demonstrated. In this connection, *E. coli* isolate showed a considerable resistance to tetracycline, trimethoprim-sulfamethoxazole, streptomycin, ampicillin, amoxicilin and erythromycin in 45-90% cases; and 77-100% isolates should a higher sensitivity to ciprofloxacin, chloramphenicol and gentamycin. The highest resistance in the present study was against tetracycline (90%) as observed in Algeria (82%) (Hammoudi and Aggad, 2008), Nigeria (88%) (Daini and Adesemowo, 2008), Iran (94.0%) (Salehi and Bonab, 2006), and Spain (95.0%) (Blanco et al., 1997). These findings confirm significant increase in the incidence of antimicrobial resistance in the *E. coli* isolates is most probably due to increased use of antibiotics as feed additives (Pelczarecs et al., 1993) for growth promotion and prevention of diseases, use of inappropriate antibiotics for treatment of diseases (Sharada et al., 2010). Moreover, it could be said that tetracycline is used frequently in poultry farms.

The resistance rates to trimethoprim-sulfamethoxazole determined in this work was relatively higher (78.5%) as compared with those found by other authors for *E. coli* isolated from poultry feces. 67% (Blanco et al., 1997) and 52.1% (Bywater et al., 2010) resistance to trimethoprim-sulfamethoxazole was determined in *E. coli* isolated from broilers in Spain. Resistance to chloramphenicol was very low (15.0%) which is comparable with other studies elsewhere including Akond et al. (2009) from Bangladesh and Sharada et al. (2010) from Ageria, who reported 20 and 21%, resistance respectively. The resistance to Ampicilin (60.0%) is in close agreement with the finding of Islam et al. (2004) and Blanco et al. (1997) who found 50% and 66% of the *E. coli* isolates were resistant to this antimicrobial. The present findings were also in partial agreement with Zinnah et al. (2008). These authors found that 80% the organisms were resistant to Erythromicin, 90% to amoxicillin and 90 % to Ampicillin.

In the present study 65% *E.coli* isolates were resistance to streptomycin. This finding was in agreement with the finding of Akond et al. (2009) from Bangladesh, who reported 70% of E.coli tested to be resistance. The current finding was also in partial agreement with that of Blanco et al. (1997) who found 81% of isolates to be resistant. In the present study there was no isolate resistant to gentamicin as was similarly reported by other studies (Salehi and Bonab, 2006; Tricia et al., 2006). A similar report of zero prevalence of resistance to gentamicin has been documented (Akond et al., 2009; Tricia et al., 2006). Nonetheless, Daini and Adesemowo (2008) reported resistance of about 54%.

In this study 38.0% of the *E. coli* isolates showed resistance against ciprofloxacin. This is higher than previous reports elsewhere such as the 10% ciprofloxacin resistance described in the Netherlands (Van Den Bogaard et al., 2001) and Bangladesh (Zinnah et al., 2008). The present finding was; however, comparable with the 38% resistance to ciprofloxacin reported from Spain (Sa´enz et al., 2001) and 33.33% resistance in Bangladesh (Nazir et al., 2005). Nonetheless, there are reports of increase in the resistance of *E. coli* to quinolones. In Bangladesh Akond et al. (2009) reported a resistance of *E. coli* in 82% isolates against ciprofloxacin. Contrary to this finding Nasrin et al. (2007) found no *E.coli* resistance against ciprofloxacin in Bagladesh layer farm, which may be due to presence of different clones of *E. coli* in the layers. One plausible explanation for this observation is the excessive use of antimicrobials for therapeutic and prophylactic treatment (Majalija et al., 2010). Furthermore, this finding showed possible abusive use of quinolones or their use as growth promoter in the poultry production in Ethiopia. As these antimicrobial agents may cause cross-resistance with human enteric pathogens (Blanco et al., 1997), prudent use of these agents in veterinary medicine is warranted.

In the present study, 93.2% of the isolates showed multiresistance at least for 2 antibiotics. Other observations also demonstrated a similar finding on multiple drug resistance of *E. coli* isolates (Khan et al., 2002; Nazir et al., 2005; Zhao et al., 2005; Salehi and Bonab, 2006; Guerra et al., 2007; Akond et al., 2009; Majalija et al., 2010). These showed administration of multiple antibiotics for prophylaxis or infection. Furthermore, it's a strong indication of abusive and indiscriminant use of antibiotics in the farms. Such multi-drug resistance may apparently be occurred which may ultimately replace the drug sensitive microorganisms from antibiotic saturated environment (Van De Bogaard and Stobberingh, 2000).

## CONCLUSION AND RECOMMENDATIONS

In general, this study showed a higher resistance rate against the antibiotics that are commonly used in poultry. There is clear evidence of abuse of antibiotics due to which emergence of multi-drug resistant *E. coli* are continuously increasing. Our field observations indicate that the abusive and anarchic use of antibiotics is probably the cause of the high percentages of resistance detected in the present study. These factors are typical of intensive poultry farming and explain the high prevalence and degree of resistance in faecal *E. coli* of poultry in this and other studies. Thus, introduction of surveillance programs to monitor antimicrobial resistance in pathogenic bacteria is strongly needed in farm and other under developing countries because in addition to animal health problems, transmission of resistant clones and resistance plasmids of *E. coli* from food animals (especially poultry) to humans can occur. Moreover, an in vitro antimicrobial susceptibility testing of veterinary pathogens that can provide valuable guidance to the veterinarian in the choice of appropriate chemotherapy should be carried out. Moreover, there should be a continuous monitoring system and rigorous sensitivity testing before application of these and other drugs singly or in combination. Since multiple drug resistance is becoming a concern both in animals and humans use of a combination of antibiotics of proven effectiveness to overcome an infection should be recommended.

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