Journal of Animal and Veterinary Advances 11 (19): 3550-3556, 2012
ISSN: 1680-5593

Isolation and Drug Resistance Patterns of *Escherichia coli* from Cases of Colibacillosis in Tabriz

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Abstract: Antimicrobial agents are used extremely in order to reducing the enormous losses caused by *Escherichia coli* infections (Colibacillosis) in Iran poultry industry. In this investigation, 200 Avian Pathogenic *Escherichia coli* (APEC) strains isolated from broiler chickens with Colisepticemia and examined for susceptibility to antimicrobials of veterinary and human significance. Multiple resistances to antibiotics were observed in all the isolates. The highest rate of resistance was against Nalidixic acid (98), Lincomycin (97.5), Erythromycin (97), Oxytetracycline (92), Chloretracycline (92), Flumequine (90), Doxycycline (80), Difloxacin (80), Neomycin (62), Streptomycin (62), Trimethoprim-Sulphamethoxazonle (60), Kanamycin (60), Enrofloxacin (60), Norfloxacin (55), Ciprofloxacin (50), Chloramphenicol (49), Furazolidone (45.5) and Nitrofurantoin (45%). Resistance to Gentamicin, Ceftifur and Fosbac were not observed and to Amikacin, Cefazolin, Colistin, Lincospectin and also Florfenicol were low. This study showed resistance rate against the antibiotics that are commonly used in poultry is very high but against them that are only used in human or less frequently used in poultry is significantly low. This study also showed that the prevalence of Quinolone Resistant *Escherichia coli* (QREC) are very high in broiler farms in Tabriz province. The high presence of OREC from broiler chickens probably id due to overuse of enrofloxacin in these farms for therapeutic purposes.

Key words: Antibiotics susceptibility, *Escherichia coli*, Colibacillosis, Nalidixic acid, Tabriz

INTRODUCTION

Bacterial infections are of worldwide importance in commercially product poultry and their costs exceed $100 million annually (Barnes *et al*., 2003). *Escherichia coli* that caused disease in chickens are collectively known as Avian Pathogenic *Escherichia coli* (APEC) (Kariuki *et al*., 2002) and have been mainly associated with extraintestinal infections such as air sacculitis, pericarditis, peritonitis, salpingitis, synovitis, osteomyelitis, cellulitis and Yolk Sac Infection (YSI) (Kariuki *et al*., 2002; Dias da Silveria *et al*., 2002; Gross, 1994).

Antimicrobial therapy is an important tool in reducing both the incidence and mortality associated with avian colibacillosis (Freed *et al*., 1993; Watts *et al*., 1993). *E. coli* may be sensitive to many antibiotics. However, isolates of *E. coli* from poultry are frequently resistance to one or more antibiotics, especially if they have been widely used in poultry industry over a long period (e.g., tetracyclines) (Watts *et al*., 1993; Allan *et al*., 1993). Antibiotics once effects at controlling *E. coli* infections are now ineffective due to the bacterium's acquired resistance to these compounds. Resistance to two or more classes of antibiotics is now commonplace in both veterinary (La-Ragione *et al*., 2004) and human (Dennesen *et al*., 1998) medicine.

Resistance generally occurs following response to prior contact with the antimicrobial but can occur naturally in the absence of orevious exposure. Resistance to florfenicol, an antibiotic related to chloramphenicol that has never been used in poultry in the United States was found in *E. coli* isolates from chickens (Keyes *et al*., 2000).

Antibiotic usage is possibly the most important factor that promotes the emergence, selection and dissemination of antibiotic-resistant microorganisms in both veterinary and human medicine (Neu, 1992; Witte, 1998). Antibiotic usage selects for resistance not only in pathogenic bacteria but also in the endogenous flora of exposed individuals (animals and humans) or populations (Piddock, 1996). Concern has been expressed about possible harmful effects on humans through the use of drugs in agriculture as follows:

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- Increased microbial
- Drug resistance drug resistance in food
- Allergic reactions and sensitization to antimicrobials
- Drug toxicity (Bazile-Pham-Khac *et al*., 1996)

Concern about antibiotic resistance and its transmission to human pathogens is important because these resistant bacteria may colonize the human intestinal tract and may also contribute resistance genes to human endogenous flora. Colonization of the intestinal tract with resistant *E. coli* from chicken has been shown in human volunteers. The result of a study was done by Van den Bogaard *et al.* (2001) strongly indicates that transmission of resistant clones and resistance plasmids of *E. coli* from poultry to humans commonly occurs (Van den Bogaard *et al*., 2001). Furthermore in some earlier studies, spread of an antibiotic resistance plasmid, pSL222-6 in *E. coli* from chickens to human handlers, finding the same O serotype in chickens from a commercial rearing centre in ovenready birds and in humans, direct transmission of *E. coli* resistant to streptomycin, sulphonamides and tetracycline from poultry to poultry attendants in Nigeria, evidence that animals are a reservoir for *E. coli* found in humans, chickens as asource of antibiotic resistance in humans in Saudi Arabia, Morocco and Northern India (Al-Ghamdi *et al*., 1999; Amara *et al*., 1995) was described. Of course in contrast, others have concluded that human and poultry isolates belong to two distinct pools of resistant *E. coli* (Bass *et al*., 1999; Caya *et al*., 1999; Kariuki *et al*., 1997; Nijsten *et al*., 1995).

On the other hand, fluoroquinolones are highly efficacious antimicrobial agents which because of their low toxicity and relatively broad-spectrum coverage are extremely valuable for treating human infections (Angulo *et al*., 2000; Livermore *et al*., 2002). In the past years they are used in treatment of certain bacterial diseases in animals, including acute bovine respiratory disease and avian colibacillosis (Hooper, 2001; White *et al*., 2000; Yang *et al*., 2004). However, a growing number of studies report an association between the emergence of fluoroquinolone-resistant zoonotic pathogens such as Salmonella, *E. coli* and Campylobacter and the subsequent approval and use of these agents in veterinary medicine (Guerra *et al*., 2003; Saenz *et al*., 2003; Yang *et al*., 2004). Garau *et al*. (1999) suggested that the high prevalence of fluoroquinolone-resistant avian *E. coli* in the stools of healthy humans in their area (Barcelona, Spain) could be linked to the high prevalence of resistant isolates in poultry and pork (Garau *et al*., 1999).

MATERIALS AND METHODS

Bacterial strains: The 200 *E. coli* isolates were recovered from clinically affected broiler chickens grown on commercial farms diagnosed with colibacillosis in Tabriz. Bacteria were originally recovered from a variety of tissues including the air sac, pericardial sac, heart, liver, trachea and blood and plated on blood agar plates and MacConkey agar. Presumptive *E. coli* isolates were confirmed using VITEK Gram-negative identification cards (BioMerieux Inc., Hazelwood, MO) following the manufacturer's instruction. Isolates were stored as 20% glycerol stocks at -70°C.

Antimicrobialsusceptibilitydetermination: Antimicrobial susceptibility determination was routinely tested by the Single-Disc Diffusion Method. The *E. coli* strains were tested against the antibiotics of human and veterinary significance. The following antibiotic discs on Mueller Hilton agar were applied: Amikacin (AN/30 μg), Cefazolin (CZ/30 μg), Chloramphenicol (C/30 μg), Chlortetracycline (CTe/30 μg), Ciprofloxacin (CP/5 μg), Colistin (CI/10 μg), Difloxacin (DIF/25 μg), Doxycycline (D/30 μg), Enrofloxacin (NFX/5 μg), Erythromycin (E/15 μg), Florfenicol (FFc/30 μg), Flumequine (FIu/30 μg), Furazolidone (FR/100 μg), Gentamicin (GM/10 μg), Kanamycin (K/30 μg), Lincomycin (L/2 μg), Lincospectin (LIN/SE), Nalidixic Acid (NA/30 μg), Neomycin (N/30 μg), Nitrofurantion (FM/300 μg), Norfloxacin (NOR/10 μg), Oxytetracycline (T/30 μg), Streptomycin (S/10 μg) and Trimethoprim-Sulphamethoxazole (SXT/25 μg).

The diameters of the zones of inhibition were interpreted by referring to the table which represents the NCCLS subcommittee's recommendation.

RESULTS

The highest rate of resistance was against Nalidixic acid (98%), Lincomycine (97.5%), Erythromycin (97%), Oxytetracycline (92%), Chloretracycline (92%), Flumequine (98%), Doxycycline (80%), Difloxacin (80%), Neomycin (62%), Streptomycin (62%), Trimethoprim-Sulphamethoxazonle (60%), Kanamycin (60%), Enrofloxacin (60%), Norfloxacin (55%), Ciprofloxacin (50%), Chloramphenicol (49%), Furazolidone (45.5%) and Nitrofurantoin (45%). Low levels of resistance were against Florfenicol (27%), Lincospectin (10%), Colistin (6%), Cefazolin (21%), Amikacin (2%), Ceftifur (0%), Fosbac (0%) and Gentamicin (0%). Susceptible (S), Intermediate (I) and Resistant (R) percentages of the isolates to the antimicrobial agents were showed in Table 1. Multiple resistances were observed in all of the isolates.

Table 1: Percentages of antibiotic susceptibility of isolated *E. coli* from broiler with colibacillosis in Tabriz province Susceptible (S), Intermediate (I) and Resistant (R) to antimicrobial agents by Disc Diffusion Method

Class and antibiotic (abbreviation)	Disc content (µg)	Diffusion zone breakpoint (mm) (NCCLS breakpoint)	S (%)	I (%)	R (%)
Fosbac	10	≤13	198	2	0
Amoxicilin (AMX)	30	≤14	9	38	53
Cephalosporins					
Cefazolin (CZ)	30	≤14	82	6	4
Ceftifur	30	≤14	100	1	0
Polymyxins					
Colistin (CI)	10	8	190	68	12
Aminoglycosides					
Amikacin (AN)	30	≤14	170	26	4
Gentamicin (GN)	10	≤12	194	6	0
Kanamycin (K)	30	≤13	60	20	120
Neomycine (N)	30	≤12	50	26	124
Streptomycin (S)	10	≤11	66	10	124
Tetracyclines					
Doxycycline (D)	30	≤12	35	5	160
Chloretetracycline (Cte)	30	≤14*	12	4	184
Oxytetracycline (T)	30	≤14	6	10	184
Nitrofurans					
Nitrofurantion (FM)	300	≤14	85	25	91
Furazolidone (FR)	100	≤14	90	20	90
Chloramphenicol (C)	30	≤12	90	12	98
Florfenicol (Ffc)	30	≤19*	132	14	54
Lincosamides					
Lincomycin (L)	2	≤14	1	4	195
Lincospectin (LIN/SE)	150	≤19*	111	9	20
Macrolides					
Erythromycin (E)	15	≤13	0	6	194
Quinolones and Fluoroquinolones					
Quinolones					
Flumequine (Flu)	30	≤19*	5	15	180
Nalidixic Acid (NA)	30	≤13	0	4	196
Fluoroquinolones					
Ciprofloxacin (CP)	5	≤15	82	18	100
Difloxacin (DIF)	10	≤17	40	3	160
Enrofloxacin (NFX)	5	≤16	68	12	120
Norfloxacin (NOR)	10	≤12	90	10	100
Sulfonamides and potentiated sulfonamides					
Trimethoprim-Sulphamethoxazole (SXT)	25	≤10	50	30	120

*No NCCLS breakpoint

DISCUSSION

The most prevalent serogroups identified among the APEC isolates were O78 (12%), O15 (5%) and O53 (4%). This is consistent with other reports that these serogroups are commonly associated with avian colibacillosis on a worldwide scale and confirms their role as particularly adapted pathogens that permit involvement in extra intestinal infections (Dho-Mainil and Fairbrother, 1999; Ewers *et al*., 2004; La-Ragione and Woodward, 2002).

Colibacillosis is the primary cause of morbidity, mortality and condemnation of carcasses in the poultry industry worldwide (Witte, 1998) and colisepticemia is the mostusual form of colibacillosis and is responsible for significant economic losses in poultry industry (Ewers *et al*., 2003). To control and prevent poultry diseases especially colibacillosis, subtherapeutic and therapeutic levels of antimicrobial agents are administered to chickens via food and water. This practice also improves feed efficiency and accelerates weight gain (Bower and Daeschel, 1999). The treatment of whole flock with antimicrobials for disease prevention and growth promotion has become a controversial practice (Amara *et al*., 1995). However, administration of antimicrobial agents provides a selective pressure which causes detection of resistant bacteria. Therefore, the antibiotic selection pressure for resistance in bacteria in poultry is high and consequently their faecal flora contains a relatively high proportion of resistant bacteria and resistance to existing antimicrobials is widespread and of concern to poultry veterinarians (Peighambari *et al*., 1995).

There is also concern that antimicrobial use in food animals can lead to the selection of antimicrobial resistant zoonotic enteric pathogens which may then be transferred to people by the consumption of contaminated food or by direct animal contact. At slaughter, resistant strains from the gut readily soil poultry carcasses and as a result poultry meats are often contaminated with multi-resistant *E. coli* (Turtura *et al*., 1990), likewise eggs become contaminated during laying. Hence, resistant faecal *E. coli* from poultry can infect humans both directly and via food. These resistant bacteria may colonize the human intestinal tract and may also contribute resistance genes to human endogenous flora. It was conclusively shown by Linton that antibiotic-resistant *E. coli* could be transferred from poultry to a food-handler's hands during food preparation and finaly to the foodstuff. The transmission of enteric bacteria to consumers via this route has been established and preventation of food poisoning is the basis for food hygiene and public health regulations in many countries (Piddock, 1996).

In this study, multiple antibiotic resistance was observed in all of the examined strains similar to the findings of earlier studies has done in Iran and other countries (Bass *et al*., 1999; Bazile-Pham-Khac *et al*., 1996; Guerra *et al*., 2003; Miles *et al*., 2006; Saenz *et al*., 2003; Salehi, 2005). Almost all the *E. coli* isolates showed high percentage of resistance to the antibiotics. High levels of resistance were against Nalidixic acid (98%), Lincomycine (97.5%), Erythromycin (97%), Oxytetracycline (92%), Chloretracycline (92%), Flumequine (98%), Doxycycline (80%), Difloxacin (80%), Neomycin (62%), Streptomycin (62%), Trimethoprim-Sulphamethoxazonle (60%), Kanamycin (60%), Enrofloxacin (60%), Norfloxacin (55%), Ciprofloxacin (50%), Chloramphenicol (49%), Furazolidone (45.5%) and Nitrofurantoin (45%). Low levels of resistance were against Florfenicol (27%), Lincospectin (10%), Colistin (6%), Cefazolin (21%), Amikacin (2%), Ceftifur (0%), Fosbac (0%) and Gentamicin (0%). So far, Tetracyclines, Enroflozacin, Streptomycin, Neomycin, Tiamulin, Flumequine and Trimethoprim-Sulphamethoxazole were extremely used in Tabriz poultry industries. For this reason, these antibiotics are inactive against avian pathogenic *E. coli* strains at the present time. Despite the fact that administration of Chloramphenicol and Furazolidone is forbidden in veterinary, resistance to this antibiotics was high. This is probably because of persistence of earlier resistances or illegal use of these agents. At the beginning of this study, resistance rate against Florfenicol (fluorinated analogue of chloramphenicol) that has been used in Tabriz poultry industries only 1 year ago was low but at the end (only 4 months later); isolation of resistant *E. coli* stains were significantly high. This event was due to extremely use of Florfenicol for treatment of the disease in poultry because of its very good effect against *E. coli*. Cefazolin and Amikacin commonly used in human but are not used in Tabriz poultry industries, also only available drug composition for Lincospectin and Gentamicin is injectable solution and they are not used as mass medication in poultry. This subject can explain the high susceptibility rate of the *E. coli* strains for these agents. Kanamycin is also used only in human but high level resistance against it is probably due to cross resistance. Kanamycin is susceptible to the largest number of enzymes but conversely, resistance to Gentamicin is mediated by modifications at few sites on the molecule. These findings confirm significant increase in the incidence of antimicrobial resistance in the *E. coli* strains is most probably due to increased use of antibiotics as feed additives for growth promotion and prevention of diseases, use of inappropriate antibiotics for treatment of disease, resistance transfer among different bacteria and possible cross resistance between antibiotics used in poultry.

Perhaps the most striking finding from this study was the widespread resistance to quinolones and fluoroquinolones. This study showed that the prevalence of Quinolone-Resistant *Escherichia coli* (QREC) is very high in the area farms. Resistance to Nalidixic acid, Flumequine, Difloxacin, Enrofloxacin, Norfloxacin and Ciprofloxacin were 98, 98, 80, 60, 55 and 50%, respectively. Although, identification of fluroquinolone-resistant avian *E. coli* isolates has been reported in other places for example in Saudi Arabia, Spain, the United States and China (Allan *et al*., 1993; Saenz *et al*., 2003; White *et al*., 2000; Yang *et al*., 2004). To the knowledge, this is the highest prevalence of resistance to quinolones in *E. coli* that has ever been reported. This high presence of QREC from the broiler chickens probably is due to overuse of enrofloxacin in this animal population for therapeutic purposes in the area. Consequently, fluoroquinolones have become infective in treatment of colibacillosis in Tabriz poultry flocks. Resistance to fluoroquinolones in these isolates, coupled with the observation of widespread multiple-antimicrobial resistance in all of the isolates.

There is mounting evidence that antimicrobial use in veterinary medicine may select for antimicrobial-resistant zoonotic bacterial pathogens (e.g., Salmonella and Campylobacter). This has led to increased pressure to limit fluoroquinolones in animals to preserve the value of these drugs in the treatment of human infections but unfortunately, there is no any program to restriction antibiotic administration in veterinary in Iran and other under developing countries (Glynn *et al*., 1998; Smith *et al*., 1999). In addition to the human health concerns, antimicrobial-resistant pathogens also pose a sever and costly animal health problem in that they may prolong illness and decrease productivity through higher morbidity and mortality (Xu, 2001). Therefore, the introduction of surveillance programs to monitor antimicrobial resistance in pathogenic bacteria strongly is needed.

The major factor selecting for antimicrobial resistance in bacteria is antibiotic use and additionally, crowding and poor sanitation. These three 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 (Van den Bogaard and Stobberingh, 1999).

Other than antimicrobials, other approaches to prevent and control APEC infections in the poultry industry include improved hygienic methods, vaccination use of competitive exclusion products and the introduction of novel immunopotentiators. However, each of these practices have had limited success (Gomis *et al*., 2003; La Ragione *et al*., 2001) and it has necessitated the use of antimicrobial chemotherapy to control outbreaks of colibacillosis. *In vitro* antimicrobial susceptibility testing of veterinary pathogens can provide valuable guidance to the veterinarian in the choice of appropriate chemotherapy. Also, research to identify new ways to minimize antimicrobial use in poultry farms is essential.

CONCLUSION

This study shows that multiple-antimicrobial-resistant *E. coli* isolates including fluoroquinolone-resistant variants are commonly present among diseased broiler chickens in Tabriz, Iran. Resistance to existing antimicrobials is widespread and of concern to poultry veterinarians. The significant increase in the incidence of resistance against antibiotics in the *E. coli* strains isolated from broiler chickens is probably due to increased use of antibiotics as feed additives for growth promotion and prevention of diseases use of inappropriate antibiotics for treatment of diseases, resistance transfer among different bacteria and possible cross resistance between antibiotics used in poultry. Thus, introduction of surveillance programs to monitor antimicrobial resistance in pathogenic bacteria is strongly needed in Iran 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. Since, the use of Cotrimoxazole and fluoroquinolones in poultry may cause cross-resistance with human enteric pathogens (especially with *Salmonella* and *Campylobacter* sp.), prudent use of these antimicrobial agents in avian species is highly recommended. To deal with multi-drug resistant organisms, it is usually recommended that potentially synergistic antimicrobial combinations be used, preventive strategies such as appropriate husbandry and hygiene, routine health monitoring and immunization should be emphasized, poultry producers should approach the treatment of diseases with antibiotics very cautiously. The producer and veterinarian should research closely when antibiotic therapy is needed in a flock and both must continue to work toward ensuring a safe food supply for consumers.

ACKNOWLEDGEMENT

Researchers would like to acknowledge the Islamic Azad University, Tabriz Branch for Financial Support of this project.

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