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Antibioresistance Profile of *Avian pathogenic Escherichia coli* Isolates Recovered from Broiler Chicken Farms with Colibacillosis in Kermanshah Province, Iran

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Abstract: Antimicrobial therapy is an important tool in reducing both the incidence and mortality associated with avian colibacillosis. Meanwhile, enormous exploitation of antibiotics in the field of poultry medicine has increased the number of resistant bacterial strains in recent years. During 2012, antibacterial susceptibility tests were performed on 154 isolates of *avian pathogenic Escherichia coli (APEC)* recovered from broiler chicken flocks with signs of colibacillosis in Kermanshah province, west of Iran. A total of 18 antibiotic discs with gentamicin, neomycin, ampicillin, chloramphenicol, florfenicol, tetracycline, chlortetracycline, doxycycline, nalidixic acid, ciprofloxacin, enrofloxacin, flumequine, norfloxacin, erythromycin, tiamulin, lincomycin-spectinomycin, colistin and fosfomycin were used. There were resistance frequencies of 91.6% and 62.3% to chloramphenicol and florfenicol, respectively. The highest frequency of resistance (97.7%) was against nalidixic acid. Frequencies of resistance to other quinolones including flumequine, enrofloxacin, norfloxacin and ciprofloxacin were also relatively high. Erythromycin and tiamulin resistance occurred at frequencies of 96.1% and 87.0%, respectively. Resistance expressed by the isolates to lincomycin-spectinomycin was at 49.4%. Relatively low fequency of resistance (30.5%) was against fosfomycin. Multi-drug resistance (MDR) was found in 63.3% of the isolates. Generally, the resistance frequencies of *APEC* isolates recovered from broiler chickens in this study were high. Studies examining isolates from various geographic locations are required to more accurately detect differences in antimicrobial resistance in isolates of *Escherichia coli* (*E. coli*). Appropriate use of antibiotics in humans and farm animals needs to be addressed in Iran and other developing countries.

Key words: *Escherichia coli* · Antimicrobial Resistance · Colibacillosis · Chickens · Iran

INTRODUCTION

Escherichia coli (*E. coli*) is one of the most important pathogens in poultry. Infections caused by *E. coli* are responsible for significant losses to the poultry industry. Colibacillosis is the most frequently reported disease in surveys of poultry diseases or condemnations at processing plants [1]. Colibacillosis refers to any localized or systemic infection caused entirely or partly by avian pathogenic *E. coli (APEC)*, including colisepticemia, coligranuloma, chronic respiratory disease, coliform cellulitis, swollen-head syndrome, coliform peritonitis, coliform salpingitis, coliform osteomyelitis/synovitis, coliform panophthalmitis and coliform omphalitis/yolk sac infection [2].

Antimicrobial therapy is an important tool in reducing both the incidence and mortality associated with avian colibacillosis [3]. Antibiotics are extensively used as productivity enhancers in poultry production or to control infectious diseases. Antimicrobial exercise and/or especially abuse are considered to be the most vital selecting force to antimicrobial resistance of bacteria [4- 7]. Antibiotic resistant pathogenic bacteria in animals can pose a risk not only to animal health, but also to humans as food-borne pathogens [8- 12]. Drug-resistant *E. coli* of animal origin may colonize the human intestine [13]. Multi drug resistant (MDR) strains of *E. coli* are commonly seen in both human and animal isolates [14]. Many bacteria have become resistant to multiple classes of antibiotics (at least three unrelated antibiotic classes)

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and deploy multiple strategies to overcome the stress of antibiotic chemotherapy. Today, MDR bacteria are causes for serious concern in hospitals and other health care institutions where they are commonly detected. Acquired MDR of *E. coli* to antibiotics creates an extensive trouble in the management of intra and extra intestinal infections, which are major causes of illness, death and increased healthcare costs [15].

Possible transfer of antibiotic resistance genes between animals and humans is a threat to public health [16]. This threat indicates stringent control measures in the use of antimicrobials in animal and human medicine. Investigation of prevalence of resistance in *E. coli* may detect possible transfer of resistant bacteria from animals to humans and vice versa. The objective of this study was to determine antimicrobial resistance among isolates of *APEC* recovered from broiler chickens with colibacillosis in western parts of Iran.

MATERIALS AND METHODS

Depending on the clinical feature of colibacillosis in chickens of each broiler farm in Kermanshah province of Iran during 2012, specimens from pericardium, air sac, infraorbital sinus, yolk sac and liver of 5 dead or euthanized sick birds per farm were cultured on blood agar and MacConkey agar plates. The broiler chickens were between 5 to 42-days of age and were raised on farms with or without recorded antimicrobial use. Farm records indicated that no antimicrobial agents were administered during the 4 days prior to sample collection. The colonies suspected to be *E. coli* were identified by standard methods [17, 18]. Antimicrobial susceptibility tests were performed on 154 isolates of *E. coli* recovered from broiler chickens with the signs of colibacillosis by the Bauer, *et al*. [19] single-disk diffusion method, in accordance with National Committee for Clinical Laboratory Standards (NCCLS), [20]. Quality control organism was used to ensure accurate performance of the tests. Most of the antimicrobial agents selected for this analysis were antibiotics that are commonly used in the treatment of colibacillosis in Iran. Although some of them are exclusively used in humans; they were assayed because they are closely related to those being used in veterinary medicine. A total of 18 antibiotic discs, shown in Table 1 were assayed. The diameter of the zones of inhibition was measured to the nearest mm and interpreted by referring to the table which represents the NCCLS subcommittee's recommendation. Data translated into sensitive, intermediate and resistant categories according to the interpretation table guidelines [21]. The antimicrobial susceptibility data was expressed as percentages of the isolates. MDR was defined as resistance exhibited to three or more antimicrobials [22].

Table 1: Percentages of *E. coli* isolates from cases of avian colibacillosis susceptible (S), intermediate (I) and resistant (R) to antimicrobial agents by NCCLS disc diffusion methods

	E. coli isolates (n = 154)		
Class and/or antibacterial agents (µg)	S	I	R
Aminoglycosides			
Gentamicin (10)	5.1	42.2	52.6
Neomycin (30)	0	31.8	68.2
Penicillins			
Ampicillin (10µg)	4.5	29.2	66.2
Phenicols			
Chloramphenicol 30)	1.3	7.1	91.6
Florfenicol (30)	25.9	11.7	62.3
Tetracyclines			
Tetracycline (30)	1.9	12.9	85.1
Chlortetracycline (30)	0.6	7.1	92.2
Doxycycline (µg)	0	20.7	79.3
Quinolones and fluoroquinolones			
Nalidixic acid (30)	0.6	0.6	97.7
Ciprofloxacin (5)	3.2	29.2	67.5
Enrofloxacin (5)	6.4	14.2	79.2
Flumequine (30)	1.3	16.8	81.8
Norfloxacin (10)	3.9	18.1	77.9
Macrolides			
Erythromycin (15)	0	3.9	96.1
Tiamulin (30)	0	12.9	87.0
Lincosamides			
Lincomycin+spectinomycin (15/200)	16.8	33.7	49.4
Polymixins			
Colistin (10)	0.6	32.5	66.9
Fosfomycin (200)	63.6	5.8	30.5

RESULTS

The percentages of susceptible, intermediate and resistant isolates to each antibiotic are outlined in Table 1. The isolates expressed resistance to almost all tested antimicrobial agents at various frequencies. The highest and lowest frequencies of resistance were against nalidixic acid and fosfomycin, respectively.

DISCUSSION

Of the aminoglycosides included in this study, resistance rates to gentamicin and neomycin were 52.6% and 68.2%, respectively. Saberfar *et al.* [23] reported low levels of resistance (12%) to gentamicin in *E. coli* isolated from cases of colibacillosis in broiler chickens during

2005-2006 in Iran. Tabatabaei and Nasirian [24] reported 48% resistance to neomycin among *E. coli* isolated from chicken flocks in Tehran, Iran. Daini and Adesemowo [25] found the resistance of *E. coli* from Nigeria in 54% strains against gentamicin. Tricia *et al.* [22] reported that no isolate of *E. coli* was found resistant to gentamicin. Although gentamicin is not legally available as a drug preparation for mass treatment of poultry flocks in Iran, relatively higher levels of resistance were found against it in this area. A commercial preparation of gentamycin plus doxycycline (Gentadox®) is illegally imported from the western borders of Iran. Administration of Gentadox® to chicken flocks by unauthorized people might have resulted in emergence of more resistant strains of *E. coli* to gentamycin in the western parts of Iran.

The isolates expressed resistance to ampicillin at a frequency of 66.2%. Resistance of *E. coli* isolates from Malaysian broiler chickens to ampicillin, with 11-95 % range has been reported [26]. Rahman *et al.* [27] reported *E. coli* isolates from broiler and layer chickens in Bangladesh were found resistant to chloramphenicol and ampicillin, in 37-87.5% of cases. Tricia *et al.* [22] reported 43 % isolates of *E. coli* were resistant to ampicillin. Akond *et al.* [28] reported that 58% of *E. coli* strains isolated from poultry and poultry environment in Bangladesh were resistant to ampicillin. Resistance rate to ampiclllin in this study was higher than those reported by Saberfar *et al.* [23] and Salehi and Bonab [29] from other parts of Iran. In recent years, ampicillin has been widely used by poultry farmers in studied area. Excessive and inappropriate treatment of diseases with ampicillin might have led to emergence of resistant *E. coli* strains.

There were resistance frequencies of 91.6% and 62.3% to chloramphenicol and florfenicol, respectively. Resistance to chloramphenicol was relatively similar to those reported by Islam *et al.* [30], Saberfar *et al.* [23] and Salehi and Bonab [29]. Using chloramphenicol is prohibited in livestock, especially poultry, in Iran. Persistence of previous resistances, illegal use of chloramphenicol, extensive use of florfenicol and likely transfer of resistant factor between the two analogues might have been the cause of high prevalence of resistant *APEC* isolates. Bacterial cross-resistance to chloramphenicol and florfenicol is being increasingly reported [11, 31-33]. Although, florfenicol is not approved for human use; it is related to chloramphenicol and can lead to occurrence of cross-resistance among bacterial pathogens.

Resistance rates among the *APEC* isolates to chlortetracycline, doxycycline and tetracycline were 92.2%, 79.3% and 85.1%, respectively. Similar results have been reported from other parts of the World [25- 30]. Salehi and Bonab [29] reported that resistance of *E. coli* isolates recovered from broiler chicken flocks with colisepticemia in Tabriz, Iran, to chlortetracycline, tetracycline and doxycycline were 95%, 94% and 88%, respectively. High rates of resistance to tetracycline (94%) and oxytetracycline (80%) among *E. coli* isolated from chicken flocks in Tehran, Iran, has been reported by Tabatabaei and Nasirian [24]. High prevalence of resistance rate of *APEC* isolates recovered from chicken flocks to tetracyclines may be the consequence of widespread and lengthy use of this group of antibiotics as feed additive, for prophylactic purposes and/or diseases treatment. Bacterial resistance to tetracycline is plasmid mediated, with a wide variety of genetic determinants [34]. This makes it more possible for a susceptible bacterium to acquire resistance factors, as was shown by Tricia *et al.* [22].

The highest frequency of resistance (97.7%) was against nalidixic acid. Frequencies of resistance to other quinolones including flumequine, enrofloxacin, norfloxacin and ciprofloxacin were relatively high (Table 1). Prevalence of quinolone-resistant avian *E. coli* (QREC) isolates has been reported in Saudi Arabia, Spain, the United States and China [9, 35- 38]. This study showed high prevalence of QREC in broiler chicken farms located in west of Iran. Similar results have been reported by Salehi and Bonab [29] from Tabriz, Iran. Tabatabaei and Nasirian [24] reported 56% and 44% resistance *in E. coli* strains isolated from chicken flocks to flumequine and enrofloxacin, respectively, in Iran in 2002. Nalidixic acid, norfloxacin and ciprofloxacin are not used in poultry flocks in Iran. High prevalence of resistant *APEC* to these quinolones in Iran may be due to widely and inappropriate use of enrofloxacin and flumequine in commercial chicken flocks. Resistance to quinolones can evolve rapidly, even during a course of treatment [39]. These findings underscore the need to monitor quinolone and fluoroquinolone-resistant bacteria in poultry production as their emergence are an important health concern.

Sensitivity rate among *APEC* isolates to lincomycin-spectinomycin was 16.8%. Saberfar *et al.* [23] reported 21% sensitivity to lincomycin-spectinomycin among the *E. coli* isolates recovered from Iranian broiler chicken flocks. Lincomycin-spectinomycin is a costly drug in Iran,

so poultry farmers do not use it readily. Appropriate and limited use of lincomycin-spectinomycin in Iran might have resulted in relatively low resistance level of *APEC* isolates to it.

The lowest rate of resistance (30.5%) was against fosfomycin; a relatively new antimicrobial in the Iranian poultry industry. Hence, it may take time to effectively select resistant bacteria.

MDR was found in 63.3% of the isolates. Similar findings have been reported before from Iran and other countries [9, 23, 27, 29, 37, 40-43]. Due to indiscriminate exploitation of antimicrobial agents, such a high incidence of MDR may be occurring which may ultimately replace the drug sensitive microorganisms with resistant ones from antibiotic saturated environment [44].

In conclusion, the resistance frequencies of *APEC* isolates recovered from broiler chickens in this study were more than those reported by other workers from some European countries [9, 45]. Possible reasons could be blind and inappropriate antimicrobial therapy, excessive use of antibiotics as feed additives for growth promotion and prophylaxis, resistance transfer among different bacteria and possible cross resistance between antibiotics used in poultry. Strategies such as strict biosecurity measures, correct management and effective vaccination programs should be employed to prevent avian colibacillosis and its predisposing diseases/factors. Appropriate use of antibiotics in humans and farm animals needs to be addressed in Iran and other developing countries. MDR is alarming and should be monitored. Introduction of surveillance programs to monitor antimicrobial resistance in pathogenic *E. coli* is strongly needed in Iran. Studies examining isolates from different geographic locations are required to more accurately detect differences in antimicrobial resistance patterns in isolates of *E. coli*.

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