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Full Length Research Paper

Antimicrobial susceptibilities of avian *Escherichia coli* isolates in Tabriz, Iran

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Colibacillosis is a poultry disease of economic importance in Iran and all around the world. The aim of this study is to test the antibiotic sensitivity of *Escherichia coli* strains which were isolated in Tabriz. A total of 100 *E. coli* strains isolated from avian colibacillosis of 50 farms from 2008 to 2009 in Tabriz, were investigated for antimicrobial susceptibility. Antimicrobial resistance of isolates was found for enrofloxacin (23%) and cefalexin (26%) as less resistant antibiotics and erythromycin (100%) and tetracycline (99%) as more resistant antibiotics.

Key words: Colibacillosis, *Escherichia coli*, Tabriz, antimicrobial susceptibility.

INTRODUCTION

Colibacillosis is the primary cause of morbidity, mortality and condemnation of carcasses in the poultry industry (Delicato et al., 2003; Dho-Moulin and Fairbrother, 1999; Ewers et al., 2004). It is an economically devastating disease for the poultry industry in the United States and many parts of the world (Dho-Moulin and Fairbrother, 1999; La Ragione and Woodward, 2002).

In the recent years, the incidence and severity of colibacillosis have increased rapidly, and current trends indicate that it is likely to continue and even become a greater problem in the poultry industry (Altekruse et al., 2002; Blanco et al., 1997). Although *Escherichia coli* is the normal microflora of the intestinal tract of chickens, a certain subset of extra intestinal pathogenic *E. coli*, termed avian pathogenic *E. coli* (APEC), possesses specific virulent attributes that have been associated with colibacillosis (Delicato et al., 2003; Dho-Moulin and Fairbrother, 1999; La Ragione and Woodward, 2002; Yang et al., 2004). Several more common reported virulent factors associated with APEC strains include increased serum survival (iss), production of aerobactin and K1 capsule, presence of type 1 and P fimbriae, and temperature-sensitive hemagglutinin (TSH) (Delicato et al., 2003; Dho-Moulin and Fairbrother, 1999; Ewers et al., 2004; Dozois et al., 1992). 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 limited success (Gomis et al., 2003; Kwaga et al., 1994; La Ragione et al., 2001; La Ragione et al., 2004; McGruder and Moore, 1999). This has made it necessary to use antimicrobial chemotherapy to control outbreaks of colibacillosis, though recent reports have shown increased resistance to antimicrobial agents commonly used for treatment (Altekruse et al., 2002; Yang et al., 2004; Bass et al., 1999; Cormican et al., 2001; Giraud et al., 2001; Vandemaele et al., 2002).

Fluoroquinolones are highly effective antimicrobial agents which are commonly used in human medicine and veterinary medicine for acute bovine respiratory diseases and avian colibacillosis (Vandemaele et al., 2002; Hooper, 2001; White et al., 2000).

However, a large 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 (Blanco et al.,

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Abbreviation: APEC, Avian pathogenic *Escherichia coli*.

Table 1. Drug resistance patterns of 100 *Escherichia coli* strains isolated from colibacillosis.

S/N	Antibiotic type	Number of experiment sample	Number of sensitive sample	Number of semi-sensitive sample	Number of resistance sample	Sensitive sample (%)
1	Enrofloxacin	100	77	19	4	77
2	Cefalexin	100	74	35	48	74
3	Neomaycin	100	58	7	36	58
4	Gentamycin	100	45	21	34	45
5	Chloramphenicol	100	30	10	60	30
6	Kanamycin	100	18	7	75	18
7	Nalidixic acid	100	1	6	93	1
8	Streptomycin	100	2	3	94	2
9	Nitrofurantion	100	16	25	59	16
10	Trimethoprim	100	16	1	83	16
11	Erythromycin	100	18	18	82	18
12	Tetracycline	100	1	10	89	1
13	Ampicillin	100	9	30	61	9
14	Cephalothin	100	17	21	5	17
15	Doxycycline	100	1	13	86	1
16	Oxytetracycline	100	5	13	82	5
17	Furazolidone	100	16	18	67	16
18	Co-amoxiclive	100	13	38	49	13

1997; Yang et al., 2004; Guerra et al., 2003; Saenz et al., 2003). Very little data though, is available on the epidemiology, prevalence or the mechanisms of antimicrobial resistance in animal feed pathogens including fluoroquinolone resistance to APEC in the United States.

MATERIALS AND METHODS

Bacterial strains

100 *E. coli* strain isolates were recovered from clinically affected broiler chickens from 50 farms diagnosed with colibacillosis in Tabriz. The bacteria were originally recovered from a variety of tissues including the air sac, pericardial sac, heart, liver, trachea, blood, and spleen and plated on blood agar plates, MacConkey agar and eosin methylene blue (EMB) agar. Presumptive *E. coli* isolates were confirmed with differential biochemical cultures such as triple sugar iron (TSI), sulfide-indole-motility (SIM), methyl red and Voges-Proskauer (MR-VP), and indole, methyl red, Voges-Proskauer, and citrate (IMVIC) test.

Antimicrobial susceptibility determination

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-Hinton agar were applied: amikacin, amoxicillin/clavulanic acid, ampicillin, cephalothin, chloramphenicol, gentamicin, kanamycin, nalidixic acid, streptomycin, tetracycline, trimethoprim, neomycin, nitrofurantion, doxycycline, furazolidone, enrofloxacin, erythromycin, nalidixic acid and cefalexin.

RESULTS

Antimicrobial resistance

All the 100 APEC isolates were tested for their susceptibility to antimicrobial agents of human and veterinary importance in accordance with antibiotic sensitivity test with antibiotic doses. APEC isolates demonstrated high rates of sensitivity to enrofloxacin (77%) cefalexin (14%) and to other antibiotics such as neomycin (58%), gentamycin (45%), chloramphenicol (30%), kanamycin (18%), nalidixic acid (1%), streptomycin (2%), nitrofurantion (16%), trimethoprim (16%), erythromycin (18%), tetracycline (1%) ampicillin (9%), cephalothin (17%), doxycycline (1%), oxytetracycline (5%), furazolidone (16%) and co- amoxiclave (13%). This is shown in Table 1.

DISCUSSION

Due to the importance of colibacillosis especially in poultry industry, compiling a protocol for remedy and control of this disease is a necessity.

In this study, the isolated *E. coli* samples from colibacillosis in Tabriz broiler farms were examined by antibiogram using antibiotics like gentamycin, nalidixic acid, chloramphenicol, kanamycin, neomycin, streptomycin, nitrofurantion, trimethoprim, erythromycin, tetracycline, ampicillin, enrofloxacin, cephalothin,

doxycycline, oxytetracycline, furazolidone, co-amoxiclave, and cefalexin. The results show that the most sensitivity is related to enrofloxacin (77%) and cefalexin (74%) and the least sensitivity is related to erythromycin (0%) and tetracycline (1%).

The least resistant drug is enrofloxacin (4%) and the most resistant drug is streptomycin (94%). The high resistance of these bacteria against tetracycline, oxytetracycline and streptomycin were reported in other researches. In one survey in Algeria on 101 *E. coli* strains, the most resistance was related to low tetracycline and streptomycin antibiotics (Ozawa et al., 2008), while in another study conducted in Trinidad from 1990 to 1997, the most resistance was related to streptomycin and tetracycline antibiotics (Lambie et al., 2000). Other studies in Canada and Morocco, verify these results are Amara et al. (1995) and Nadeau et al. (2000).

The results from the broiler farms in Tabriz indicated resistance to tetracycline, streptomycin, nalidixic acid and some of the sulphonamides that were expanded may be related to bacterial properties and irregular usage of antibiotic drugs. In one survey in 2004 done by Zhao et al. in the United States on 95 *E. coli* strains from colibacillus, using antibiogram experiment, the most resistance was against tetracycline and streptomycin while the least resistance was against enrofloxacin, in accordance with present results. However, about 69 and 59% resistance to the antibiotics gentamycin and nalidix acids respectively were reported, contrary to our results (Zhao et al., 2005). The difference in antibiogram results may be related to the properties of pathogenic bacteria in any region and to the difference in the rate and usage of antibiotics. Ozawa et al. in 2001 to 2006 studied 83 *E. coli* strains from colibacillosis in Japan and reported that the most resistance was against ampicillin and oxytetracycline while the least resistance was against enrofloxacin and florfenicol (Ozawa et al., 2008).

Finally, these studies' results and the other results were compared and show that very high resistance to some common antibiotics in poultry farms may cause problems of usage of these antibiotics, because they do not have remedial effects, increase economic costs and cause side effects of antibiotics abuse and difficulties when antibiotic drugs like tetracycline, erythromycin, nalidixic acid and doxycycline remain in the flesh. These results show the need for using multi-antibiotic remedial protocols for effective therapy of colibacillosis in Tabriz.

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