Isolation, Identification and *in vitro* Susceptibility of Avian *Escherichia coli* to Selected Fluoroquinolones

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Summary

50 clinical *Escherichia* coli were isolated from 51 broiler farms in Urmia. An evaluation on antibacterial susceptibility testing of them against three selected fluoroquinolones was conducted. By using the Mast Diagnostics kit, the O1, O6, O15 and O55 serotypes were determined. For flumequine, enrofloxacin and norfloxacin the MIC values were 97.8, 12.6, and 24.4 μg/ml, respectively. Of the tested bacteria 8.6%, 34.6% and 24.4% were sensitive and 67.5%, 34.8% and 20.4% were resistant to flumequine, enrofloxacin and norfloxacin, respectively.

***Key words*:** broiler, fluoroquinolones, enrofloxacin, flumequine, norfloxacin, *E.coli*

Introduction

Escherichia coli (*E.coli*) is one of the normal bacterial floras in the gastrointestinal tract of poultry. 10-15% of the intestinal coliforms in chickens are of pathogenic serotypes. Colisepticemia, respiratory tract infections, poultry cellulite, swollen head syndrome, omphalitis/yolk-sac infection, pericarditis, peritonitis and salpangitis are important diseases caused by *E.coli* in birds (Barnes 1997). Although the killed (O2:K1 and O78:K80) and lived (BT-7) vaccines have been produced to immunize chickens against pathogenic *E.coli* antibacterial compounds are widely used for prevention, control and treatment of the *E.coli* born conditions (Barnes & Gross 1997, Falkow & Kalanos 1990).

Quinolones are a group of synthetic antibacterial agents, which were introduced 30 years ago. These compounds possess bactericidal effect against gram negative organisms and initially were employed to control urinary infections (Prescott 1993). Flumequine, a first-generation fluoroquinolone, is an older member of this group, which is still used for the treatment of enteric as well as systemic infections. Due to the occurrence of resistance and to their considerable toxicity, particularly in the

central nervous system the application of quinolones has been largely hindered. Fluoroquinolones, which have replaced early quinolones, are newer compounds with more potent antibacterial activity, higher tissue distribution and wider spectrum of activity. The main toxicity of these substances is damage to weight-bearing joints when used at trapeutic doses in some immature animals. These agents interfere with the function of the bacterial DNA gyrase, hence inhibiting the DNA synthesis during replication (Lesse 1995). Enrofloxacin, norfloxacin, danofloxacin, and ciprofloxacin are the most commonly used fluoroquinolones in veterinary practice (Prescott & Baggot 1993, Barragy 1994, Lesse 1995, Spoo & River 1995). These groups of drugs commonly use in Iran to control or to treat poultry diseases caused by *E.coli*. In the present study the antibacterial susceptibility of this microorganism, isolated from chickens with colibacillosis, to three selected fluoroquinolones were evaluated.

Materials and Methods

Test organisms. 51 clinical bacteria isolates were selected for testing. The collection included *E.*coli sp. (50 isolates) and Citrobacter sp. (1 isolate). The test organisms were all recent clinical isolates contributed by 51 broiler farms in Urmia. They were from hearts, livers and air sacs. The isolates were cultured on MacConkey agar medium (Difco) and incubated overnight at 37C. The colonies that suspected to be *E.coli* were identified by standard methods (Holt *et al* 1996).

Serotype identification. The Mast Diagnostics Kit® (Mast Group Ltd., Merseyside, UK) was used to identify of *E.*coli. Using polyvalent antisera the agglutination test was performed on the isolated bacteria. When the bacteria were agglutinated within 1 min, using the relevant antiserum, they were assayed again for precise identification of the O antigens.

K antigens, which mask the O complex, are found in *E.coli*. According to manufacturer instruction suspension of the bacteria that fail to agglutinate in O antiserum, was heated at 100C for 60 min, and retest in the appropriate O antisera.
When both live and killed bacteria reacted positively with a specified monovalent antigen, the bacteria were identified as pathogenic *E.coli*. The poly- and monovalent antisera in Mast Diagnostic kit were shown in table 1.

Antibacterial drugs and dilution trays. Flumequine (Damloran Pharmaceuticals, Iran), enrofloxacin (Science Laboratory, Iran) and norfloxacin (S.P. Veterinaria, Spain) were obtained from their respective manufactures. Dilution trays containing serial dilutions of the antibacterial agents were prepared in Muller-Hinton broth medium.

Table 1. *Polyvalent and monovalent antisera in Mast Diagnostic kit*

Polyvalent 1	O1, O26, O36a, O111, O119, O127a, O128
Polyvalent 2	O44, O55, O126, O146, O168
Polyvalent 3	O18, O114, O142, O151, O157, O158
Polyvalent 4	O6, O27, O78, O148, O159, O168
Polyvalent 5	O20, O25, O63, O153, O167
Polyvalent 6	O8, O15, O115, O169
Polyvalent 7	O28ac, O112ac, O124, O136, O144
Polyvalent 8	O29, O143, O162, O164

The final concentration ranges of the antibacterial agents were 0.25-256μg/ml. Each tray also contained a positive growth control tube without an antibacterial agent.

Antibacterial susceptibility test method. Broth serial dilution testing was performed according to Hindler and Jorgensen (1995) instruction. An inoculum concentration containing approximately 10^5 to 10^6 organisms/ml was prepared from an *E.coli* pure culture plate in Muller Hinton broth. The tube was incubated overnight at 37C then the turbidity was adjusted to the turbidity of a 0.5 McFarland standard. In each tray the concentration of drugs were from 0.125 to 128μg/ml. The positive growth control (broth plus inoculum) and a tube containing broth alone were set up to serve as a negative control. The trays were incubated at 37C for 24h. The MIC was defined as the lowest concentration of antibacterial drug showing no growth.

Results and Discussion

All isolated and identified bacteria possessed the morphological and biochemical characteristics of *E.coli*, except in one case that was identified as *Citerobacter sp*. In serotype identification test, the serotypes of isolated bacteria were found to be O1, O6, O15, and O55. It should be declared that serotyping was not possible for a number of isolated *E.coli* with the used kit.

Table 2 summarizes the susceptibilities of 50 *E.*coli isolates to flumequine, enrofloxacine and norfloxacin. The O1 and O55 serotypes are known as pathogenic in poultry and are usually isolated from birds with colibacillosis (Gross *et al* 1988, Gyimah & Panigrahy 1988, Panigray & Ling 1990, Allen *et al* 1993, Singh *et al* 1997). Isolation of the O6 and O15 serotypes, which usually cause septicemic diarrhea in newborn and enteritis in domestic animals is an evidence that the water sources of the farms were probably contaminated with sewage and/or the farm laborers did not observe sanitary measures (Kim & Namgoong 1987, Bole-Hribovsek *et al* 1988, Blanco *et al* 1993, Jingyu & Guoxiang 1996).

Table 2. *The MIC of flumequine, enrofloxacin and norfloxacin against avian E.coli*

Antimicrobial agent	n	MIC(µg /ml)			Susceptibility (%)			
		Range	Min	Max	Comp.	Mod.	Inter.	Resis.
Flumequine	50	0.125-128	12.12	128	8.6	-	23.9	67.5
Enrofloxacin	50	0.125-128	1.26	32	34.6	-	30.6	34.8
Norfloxacin	50	0.125-128	1.08	32	24.4	14.4	40.8	20.4

n=number of tested bacteria; Comp.=complete susceptible; Mod.=moderately susceptible; Inter.=intermediately susceptible; Resist.=resistant

In this survey, only 8.6%, 24.4%, and 34.6% of the tested specimens were found to be sensitive to flumequine, norfloxacin, and enrofloxacin, respectively (Table 2). As shown in table 2, the fluoroquinolone-resistant *E.coli* existed in the farms. The highest incidence of resistance (67.5%) was observed against flumequine, one of the oldest fluoroquinolones used commercially. Because of a limited tissue diffusion capability, a narrow antibacterial spectrum, and a lower antibacterial potency (compared with new-generation fluoroquinolones) this drug unable to exert its antibacterial action on avian *E.*coli strains. In addition, acquired resistance develops readily against flumequine. Nalidixic acid, another antibacterial with structural similarity, has also been shown to share these characteristics (Barragry 1994, Spoo & River 1995, Blanco *et al* 1997, Sujen & Chingho 1997). The effect of enrofloxacin on tested bacteria was more than norfloxacin. It may be attributed to the higher bioavailability and potency of enrofloxacin (Prescott & Baggot 1993, Abd el-aziz *et al* 1997, Sumano *et al* 1998).

An important finding of our study was the resistance of a large number of isolated *E.coli* to three employed antibacterials. Chromosomal alteration is suggested to be the main mechanism of resistance against fluoroquinolones. Through this mechanism, mutation alters the genes, which code for DNA gyrase (A and B subunits) leading to a decreased affinity of the enzyme to fluoroquinolones. Resistance may also occur due to alterations in the bacterial cell wall and, hence, a decreased permeability to fluoroquinolones (Prescott & Baggot 1993, Barragry 1994, Spoo & River 1995). On the other hand, cross resistance among these antibacterial agents has been also reported. It is possible that the bacteria, which have already acquired resistance against quinolones show resistance to fluoroquinolones, too (Prescott & Baggot 1993, Sumano *et al* 1998).

Although fluoroquinolones are the most effective antibacterial agents used in poultry industry, performing antibacterial susceptibility tests may be a reliable guide to select a suitable antibacterial agent. Besides, application of recommended dosage regimens and duration of therapy, as well as elimination of older quinolones from pharmacopoeias may decline the extent of bacterial resistance to fluoroquinolones and enhance the time span that these agents will be used.

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