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Isolation, Identification and Antimicrobial Resistance Patterns of E. coli Isolated from Chicken Flocks

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ABSTRACT

Fifty E. coli strains isolated from chicken flocks were analyzed to determine their resistance to antimicrobial agents used in Tehran poultry industry. By using Mast Diagnostic kit only O6 serotype was identified. Multiple resistance to antibiotics was observed in all isolates. The highest rate of resistance was against Tetracycline (94%), followed by Rifampicin (90%), and Oxytetracycline (80%). Fifty E. coli isolates showed 10 different patterns of resistance to the antimicrobial agents used in this study. The most common antimicrobial resistance pattern of these isolates was Enrofloxacin / Trimethoprim-Sulphamethoxazole / Tiamulin / Flumequine / Sulphadiazine / Oxytetracycline / Rifampicin. The present study confirms significant increase in the incidence of resistance in E. coli isolated from poultry which is probably due to increased use of antibiotics as feed additives for growth promotion and prevention of disease, resistance transfer among different bacteria and possible cross resistance between antibiotics used in domestic animals and those used in human medicine.

Keywords: *E. coli, Poultry, Antibiotic-Resistance*

Escherichia coli is one of the normal bacterial flora of the gastrointestinal tract of poultry. Ten to fifteen percent of the intestinal coliforms in chickens are of pathogenic serotypes [1]. Colibacillosis is a common systemic infection caused by E. coli in poultry. The disease causes considerable economic damage to poultry production world wide [2].

Significant increase in appearance of drug-resistant strains of E. coli isolated from poultry has complicated the problem [3-6]. In this study, E. coli strains isolated from poultry were analyzed to determine their susceptibilities to antimicrobial agents used in Tehran poultry farms.

MATERIAL AND METHODS

Bacterial strains

A total of 50 strains of E. coli were isolated from bile and liver of poultry in several aviaries, between October 2001 till January 2002 in Tehran.

Bacteriology

Preliminary bacteriological assays for all specimens were carried out. Specimens were cultured on McConkey and EMB agar. The colonies suspected to be E. coli were identified by standard methods [7]. Final identification of isolated strains was performed by agglutination test using the Mast Diagnostic kit (Mast Group Ltd., Merseyside, UK). 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 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 bacteria that fail to agglutinate in O antiserum was heated at 100°C for 60 min, and retested with 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 are shown in (Table 1) [6, 7].

Antimicrobial resistance pattern

All isolates were routinely tested by the single-disk diffusion method [7]. Mueller Hinton Agar was prepared according to the manufacturer's directions (Difco). With a sterile wire loop, the tops of four or five isolated colonies of a similar morphologic type were transferred to a tube containing 4 to 5 ml of suitable broth medium. The broth was incubated at 35°C until its turbidity exceeded that of the MCFarland 0.5 barium sulfate standard. Within 15 minutes of adjusting the density of the inoculum, a sterile cotton swab on a

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

Polyvalent	Monovalents
Polyvalent 1	O_1, O_{26}, O_{36a}, O_{111},O_{119}, O_{127a},O_{128}
Polyvalent 2	O_{44}, O_{55}, O_{126}, O_{146}, O_{168}
Polyvalent 3	O_{18}, O_{114}, O_{142}, O_{151}, O_{157}, O_{158}
Polyvalent 4	O_6, O_{27}, O_{78}, O_{148}, O_{159} ,O_{168}
Polyvalent 5	O_{20}, O_{25}, O_{63}, O_{153}, O_{167}
Polyvalent 6	O_8, O_{15}, O_{115}, O_{169}
Polyvalent 7	O_{28ac}, O_{112ac}, O_{124}, O_{136}, O_{144}
Polyvalent 8	O_{29}, O_{143}, O_{162}, O_{164}

Table 2. Antimicrobial resistance patterns of E. coli isolates.

No. of resistance patterns	Resistance patterns
1	Enro, Sxt, Tia, Flu, Sul, Otet, Rif
2	Chlor, Rif, Neo, Strep, Enro, Sul
3	Enro, Strep, Neo, Tia, Sxt
4	Rif, Tet, Tia, Neo, Kan, Enro
5	Otet, Strep, Ceph, Rif, Tet, Neo
6	Neo, Rif, Amp, Chlor
7	Otet, Sxt, Neo
8	Amp, Chlor, Enro
9	Sul, Amp, Chlor, Kan
10	Amp, Chlor

wooden applicator stick was used to streak the dried surface of Mueller-Hinton plates in three different planes. The inoculated plates were allowed to remain on a flat surface 3 to 5 minutes for absorption of excess moisture, and then the disks were applied. Selected disks for our study were: Enrofloxacin (Enro/5 µg), Trimethoprim-Sulphamethoxazole (Sxt/30 µg), Tiamulin (Tia/30 µg), Flumequine (Flu/30 µg), Sulphadiazin (Sul/400 µg), Oxytetracycline (Otet/30 µg), Rifampicin (Rif/30 µg), Chloramphenicol (Chlor/30 µg), Neomycin (Neo/30 µg), Streptomycin (Strep/10 µg), Tetracycline (Tet/30 µg), Kanamycin (Kan/30 µg), Cephalotin (Ceph/30 µg) and Ampicillin (Amp/10 µg). After 16 to 18 hours incubation, each plate was examined, and the diameters of the complete inhibition zones were noted and measured. The diameters of the zones of inhibition were interpreted by referring to the table which represent the NCCLS subcommittee's recommendation [8].

RESULTS

All isolated and identified bacteria possessed the morphological and biochemical characteristics of E. coli. Using polyvalent and monovalent antisera in Mast Diagnostic Kit only O_6 serotype was determined. It should be declared that serotyping was not possible for a number of isolated strains with the used kit.

All isolated E. coli showed resistance to two or more antibiotics, so multiple resistance was observed in all of them. The highest rate of resistance was against Tet(94%), followed by Rif (90%), Otet (80%), Tia (76%), Chlor (58%), Flu (56%), Sul (56%), Sxt (50%), Strep (48%), Neo (48%), Kan (46%), Enro (44%), Ceph (34%) and Amp (28%) (Fig 1). Fifty E. coli isolates showed 10 different patterns of resistance to the antimicrobial agents used in this study (Table 2). The most common antimicrobial resistance pattern of these isolates was Enro/Sxt/Tia/Flu/Tet/Otet/Rif (28%) followed by Neo/Rif/Amp/Chlor (18%) and Chlor/Rif/Neo/Strep /Enro/Sul (14%) (Fig 2).

DISCUSSION

Although the killed and live vaccines have been produced to immunize chickens against pathogenic E. coli, Colibacillosis is one of the most common bacterial infections in the poultry [1, 6]. The serotypes O_1 and O_{55} are known pathogens in poultry and are usually isolated from birds with Colibacillosis [9-14]. Isolation of O_6 serotypes which usually cause septicemic diarrhea in newborn and enteritis in domestic animals is evidence that the water sources of the farms were probably contaminated with sewage and/or the farm laborers did not observe sanitary measures [10, 15-18].

In our study, all E. coli isolates showed high percentage of resistance to drugs used and multiple drug resistance was observed in all studied strains. In this study, resistance against Tet, Otet, and Neo was 94%, 80%, and 48% respectively. Other workers reported that 90% of E.coli strains isolated from poultry, were resistant to the two tetracyclines (chlortetracycline and oxytetracycline), and 20% of studied strains were resistant to Neo [3]. Resistance against Enro and Flu was 44% and 56% respectively, which was almost similar to those previously reported from Iran (34.8%, 67.5%) [6]. Other workers reported multiple drug resistance in E. coli strains isolated from poultry in Iran, and it was revealed that all of the cultures were resistant to Tet, Amp, Strep, and Sulfoamide [4]. In a previous study, it was showen that a high percentage of E. coli (86.5%) isolated from avian feces were resistant to one or more antibiotics [19]. To control and prevent poultry diseases, breeders administer subtherapeutic and therapeutic levels of antimicrobial agents to chickens via food and water. This practice also improves feed efficiency and accelerates weight gain [20]. Administration of antimicrobial agents to poultry, however has provided a selective pressure which explains the detection of resistant bacteria and as a result, many bacteria associated with poultry product are commonly resistant to antimicrobial agents [5, 20-23]. Food and drug administration (FDA) has emphasized the problem that antibiotics-fed animals frequently present resistant strains of Enterobacteriaceae that increase the spread of drug resistance to human. In fact these resistant strains could colonize, though transiently, in the human gut and/or transfer their R-factor (Resistance transfer factor) to the intestinal flora of human. A great similarity between the plasmids of Enterobacteriaceae isolated from animal and humans has been observed [24-26]. Other workers reported that transmission of resistance plasmids of E. coli from poultry to human commonly occurs [27]. Previous studies have revealed that the use of fluoroquinolones in poultry industry, is thought to be inappropriate, due to cross resistance with fluoroquinolones used to treat important human enteric infections, and high level of resistance to chlortetracycline and oxytetracycline is of concern due to possible cross resistance with antibiotics used in human medicine and there is a link between the use of antimicrobial agents in poultry and other food producing animals, and the emergence of human pathogens with decreased susceptibilities or complete resistance to

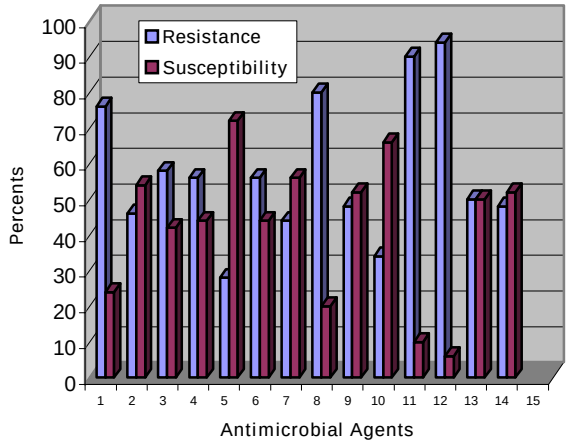

Fig 1. Resistance and Susceptibility of E. coli isolates againts the used antibiotics. 1=Tiamulin; 2=Kanamycin; 3=Chloramphenicol; 4=Flumequine; 5=Ampicillin; 6=Sulphadiazine; 7=Enroflozacin; 8=Oxytetracycline; 9=Streptomycin; 10=Cephalotin; 11=Rifampicin; 12=Tetracycline; 13=Trimethoprim-Sulphamethoxazole (SXT); 14=Neomycin.

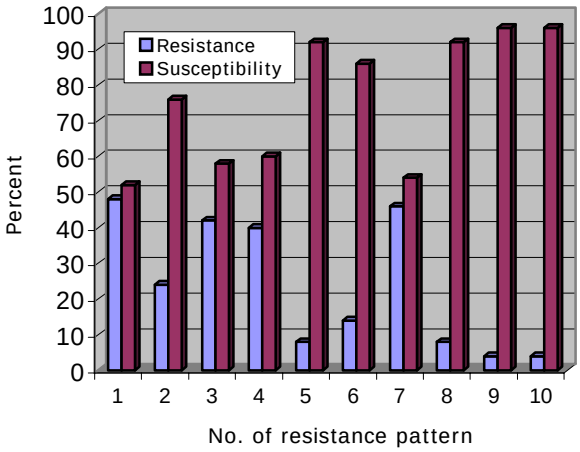

Fig 2. Percent of antimicrobial resistance patterns in E. coli isolates.

antibiotics used for treatment of human infections [28-31]. In a study, high rate of resistance to Sxt and the presence of multidrug-resistant strains in E. coli isolated from urinary infections in the southeast of Iran are reported [32]. Another study has revealed that out of 90 Enterobacteriaceae isolated from the cases of urinary tract infection, 95.5% were resistant to Amp, Tet, Chlor, Trimethoprim and Sulphamethoxazole [33]. These data confirm that significant increase in appearance of drug resistant strains in poultry is due to uncontrolled use of antimicrobial agents as food additives, for therapy and control of bacterial infections. Transfer of R-factor to the intestinal flora of human and possible cross resistance between antibiotics used in domestic animals and those used in human medicine, result in the emergence of human pathogens with resistance to antibiotics used for treatment of human infections.

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