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Antimicrobial Drug Resistance Pattern of *Escherichia coli* Isolated from Chickens Farms with Colibacillosis Infection

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Abstract

Colibacillosis refers to any localized or systemic infection caused entirely or partly by avian pathogenic *Escherichia coli* (APEC). Colibacillosis in mammals is most often a primary enteric or urinary tract disease, whereas colibacillosis in poultry is typically a localized or systemic disease occurring secondarily when host defenses have been impaired or overwhelmed by virulent *E. coli* strains. The purpose of this study was to investigate the antimicrobial drug resistance pattern of *Escherichia coli* isolated from broiler chickens farms with colibacillosis infection. Dead birds from commercial broiler chicken farms showing signs of colibacillosis were necropsied and swab samples were collected from internal organs and blood aseptically for the isolation of *Escherichia coli*. Pure colonies of the bacteria were isolated on solid media and the isolates were identified as *E. coli* based on morphological and biochemical characteristics. For determination of susceptibility to antibacterial agents, the disc diffusion method on Muller-Hinton agar was used. The following antimicrobial agents were tested: gentamycin, oxytetracyline, colistin, ciprofloxacin, doxycycline, nalidixic acid, co-trimoxazole (trimethoprim-sulfamethoxazole), norefloxacin, lincospectin and cefuroxime. The drug resistance patterns of the organisms were determined as a percentage and reported at three levels: susceptible, intermediate and resistant. All the isolates of *Escherichia coli* showed resistance to several antibiotics and a pattern of multiple drug resistance was observed. The highest rate of resistance was observed against nalidixic acid (100%) and the least rate of resistance was observed against gentamycin (17%). According to the results of this research care must be taken to avoid secondary infection (colibacillosis) in chicken farms and also avoid in careless antimicrobial consumption in food animals including chickens.

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Keywords

Chickens, Colibacillosis, Drug Resistance, *Escherichia coli*

1. Introduction

Infection caused by *Escherichia coli* is known as colibacillosis. Airsacculitis, pericarditis, peritonitis, salpingitis, synovitis, osteomyelitis, cellulitis, coligranuloma, colisepticaemia and yolk sac infection are syndrome of *E. coli* infection [1]. *Escherichia coli* is the type genous of the family Enterobacteriaceae and grows aerobically or anaerobically on ordinary nutrient media at temperature 18˚C - 44˚C. It ferments carbohydrates, often producing gas [2]. Organisms are easily isolated and identified for confirmation of the disease. All broad-spectrum antibiotics respond to the infection [3]. Isolation of *E. coli* is significant if made from the internal organs or blood from fresh carcasses [4]. *Escherichia coli*, *Enterobacter*, and *Klebsiella* ferment lactose and can be distinguished from most of the other enteric organisms [4] [5]. Most avian pathogenic *Escherichia coli* (APEC) isolated from poultry are specific clonal types that are pathogenic only for birds and represents a low risk of disease for people or other animals. However, chickens are readily infected experimentally with *E. coli* O157:H7, an important enterohemorrhgic pathogen of humans that produces Shiga toxin, and can shed the organism for months. Natural infection with O157:H7 has been identified in both chickens and turkeys in different geographic areas. Contamination of poultry meat with this organism can occur as evidenced by a food-borne outbreak of diarrheal disease that was associated with contaminated turkey meat. Poultry, especially pigeons in certain geographic areas, are a natural reservoir for Shiga toxin producing *E. coli* (STEC) which is a potential health hazard to people [2]. Various antimicrobials in intensively managed food animals including chickens are often administered through feed or drinking water either for therapy, prophylaxis, or growth promotion [6]. High prevalence of resistance to antimicrobial agents amongst isolates of *E. coli* isolated from chickens has been reported [7]-[9].

The objectives of the present study were to investigate the antimicrobial resistance pattern of *Escherichia coli* isolated from broiler chickens farms with colibacillosis infection.

2. Materials and Methods

Dead birds from commercial broiler chickens farms in Zabol, southeast of Iran, showing signs of *Escherichia coli* infection (colibacillosis) were necropsied and swab samples were collected from internal organs and blood aseptically for the isolation of *Escherichia coli*. Swab samples were collected in 5 ml Tryptone Soya Broth (TSB) and were transferred to the laboratory of microbiology and were incubated at 37˚C for 18 - 24 hours and were investigated for the presence of *Escherichia coli*. A total of 100 *E. coli* were isolated from chickens with fibrinous prehepatitis, precarditis, airsaculitis and yolk sac infection. Pure cultures were prepared from *E. coli* like colonies on Mac Conkey agar and Eosin Methylene Blue (EMB) agar (Merck, Germany). All isolates were identified as *E. coli* based on morphological and biochemical characteristics.

Antimicrobial Susceptibility Testing

For determination of susceptibility to antibacterial agents, the disc diffusion method on Muller–Hinton agar was used. Some colonies of isolated bacteria from an agar plate were transferred to a glass tube containing 5 ml saline and the suspension was vortexed and visually matched with 0.5 MacFarland standard for turbidity. Sterile cotton tipped swab was dipped into the prepared bacterial suspension and then rolled on the upper part of the tube to remove the excess fluid and subcultured onto Muller-Hinton agar [10].

Paper discs with predetermined amounts of antibiotics were used. The following antimicrobial agents were tested: gentamycin (10 µg), oxytetracyline (30 µg), colistin (10 µg), ciprofloxacin (5 µg), doxycycline (30 µg), nalidixic acid (30 µg), co-trimoxazole (trimethoprim-sulfamethoxazole) (1.25/23.75µg), norefloxacin (10 µg), lincospectin (15/200µg) and cefuroxime (30 µg). All antibacterial discs were provided from Padtan Teb Company (Tehran, Iran). Following the application of antimicrobial discs, the plates were incubated at 37˚C for 24 hours.

The diameters of the zones of inhibition were measured (millimetres) and were compared to internationally

accepted measurements to determine the susceptibility or resistance of the isolates. Results were interpreted to the Clinical Laboratory Standard Institute (CLSI) guidelines [10]. Drug resistance patterns of the organisms were determined at three levels: Susceptible (S), Intermediate (I) and Resistant (R). The numbers of isolates of *Escherichi coli* which showed S, I and R patterns were determined. The percentages of antimicrobial resistance of each pattern (S, I and R) of isolates were calculated and reported as the results.

3. Results

All the isolates of *Escherichia coli* showed resistance to many antibiotics and a pattern of multiple drug resistance was observed. The rates of resistance against nalidixic acid (100%), colistin (99%), oxytetracycline (96%), doxycycline (95%), ciprofloxacin (91%), trimethoprim-sulfamethoxazole (89%), norefloxacin (88%), lincospectin (53%), cefuroxime (50%) and gentamycin (17%) were observed.

The rastes of susceptibility against gentamyci (81%), lincospectin (41%), cefuroxime (16%), trimethoprim-sulfamethoxazole (10%), norefloxacin (9%), ciprofloxacin (7%), oxytetracycline and doxycycline (3%), colistin and nalidixic acid (0%) were observed. Antibacterial resistances pattern of isolated *Escherichia coli* are shown in **Table 1**.

4. Discussion

Antibiotic susceptibility pattern of *Escherichia coli* isolated from chickens showed highest resistance to nalidixic acid (100%) and followed resistace to colistin (99%), oxytetracycline (96%), doxycycline (95%), ciprofloxacin (91%), co-trimoxazole (89%), norefloxacin (88%), lincospectin (53%), cefuroxime (50%) and gentamycin (17%) were observed. In the area of this study respiratory viral diseases of broilers like Newcastle disease and infectious bronchitis occurs and subsequently secondary bacterial diseases like *Escherichia coli* infection are frequently observed. For treatment of secondary infection generally antibiotics are used with or without antibiogram testing. Besides, two compounds of antibiotics for treatment of secondary infection are usually administrated. The following compounds of antibiotic are almost used: polypeptides, lincosamides, macrolides, aminoglycosides, tetracyclines, sulfonamides, fluoroquinolones and pleuromutilines. This abuse consumption of antibiotics produced a wide range of antibiotic resistance. Moniri and Dastehgoli (2007) investigated the antimicrobial resistance pattern of *Escherichia coli* isolated from chickens in Iran. They observed a high level of resistance among the *Escherichia coli* as following: trimethoprim-sulfamethoxazole (98.7%), ciprofloxacin (69.7%), doxycycline (99.4%) and nalidixic acid (99%) [9]. The results of Moniri and Dastehgoli (2007) are similar to our finding in this study. Antibiotics are used as feed additives to improve feed efficiency and weight gain [11]. Many antibiotics are also used in feed and water to control disease. Indiscriminate use of antibiotics has provided selective pressure for the emergence of drug resistant strains of bacteria associated with poultry products [11]-[13]. Transmission of the R-plasmid from *E. coli* of poultry to human occurs very commonly [14]. Earlier studies revealed that use of fluoroquinolones in poultry was not appropriate due to the cross-resistance with fluoroquinolones used to treat human enteric infections. High resistance to chlortetracycline and oxytetracycline is of major concern because of the use of the same antibiotics in human medicine and poultry or in other food animals and the emergence of drug-resistant human pathogen [15] [16]. Antibiotic resistance of fecal *E. coli* was greater in broilers and turkeys that received antibiotics relatively frequently compared to layers, which had little exposure to antibiotics [14]. Similar antibiotic resistance patterns were present in *E. coli* isolated from people who worked with these birds, and in some instances specific strains were shared among the birds and workers in-

Table 1. Antibacterial resistance patterns among *Escherichia coli* isolated from chickens with colibacillosis (100 isolates).

	Antibacterial agent									
Results	GM10	T30	CL10	CP5	D30	NA30	SXT	NOR10	LP	XM30
S (%)	81	3	0	7	3	0	10	9	41	16
I (%)	2	1	1	2	2	0	1	3	6	34
R (%)	17	96	99	91	95	100	89	88	53	50
Total (%)	100	100	100	100	100	100	100	100	100	100

GM10, gentamycin; T30, oxytetracyline; CL10, colistin; CP5, ciprofloxacin; D30, doxycycline; NA30, nalidixic acid; SXT, co-trimoxazole (trimethoprim-sulfamethoxazole); NOR10, norefloxacin; LP, lincospectin; XM30, cefuroxime; S, susceptible; I, intermediate; R, resistant.

dicating that transmission of resistant organism and/or plasmids from poultry to people occurs commonly [14] [17].

5. Conclusion and Recommendations

Broiler chickens are main sources of protein for humans and broilers are very susceptible to *Eschericia coli* infection. Good management should be prepared for chickens farms to avoid secondary bacterial infection. *Escherichia coli* isolated from cases of colibacillosis showed resistant to many antibiotics. Indiscriminate use of antibiotics in food animals including chickens should be avoided to decrease drug resistant *Eschericia coli* strains.

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