



Short Communication

Determining the antibacterial resistance pattern of *Escherichia coli* isolated from poultry infected with colibacillosis in Kohgiluyeh and Boyer-Ahmad province of Iran

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ABSTRACT

One hundred and fifty samples suspected of coli bacillosis were isolated from broilers; laying hens and mother hens were sampled from 21 farms. The test was positive in 98 cases. And their sensitivity to ten types of antibiotics was investigated using antibiogram method, the disc diffusion method and the growth inhibition halo diameter in (mm). Ninety positive cases (60%) were meat breeding chickens and 8 positive cases (5.33%) were mother and laying hens. The antibiotic discs were related to tetracycline, ampicillin, difloxacin, enrofloxacin, doxycycline, sulfamethoxazole + trimethoprim, ciprofloxacin, calcitin, florfenicol, Fozbak. The biggest halo was related to florfenicol and Fosbak (21-22 mm). Also, the smallest halo was related to tetracycline and ampicillin (8-9 mm). Average halo diameter among ten antibiotics was measured from less than 8 mm to more than 22 mm. . All isolated *Escherichia coli* were at least 26% resistant to 7 types of antibiotics. Old generation antibiotics such as tetracycline and ampicillin were less effective than new generation antibiotics. In conclusion, the frequency of antibacterial resistance was high due to their excessive consumption and it would be a serious risk for the country's poultry industry and public health.

تعیین الگوی مقاومت ضد باکتریایی اشریشیا کلی جدا شده از طیور آلوده به کلی باسیلوز در استان کهگیلویه و بویراحمد، ایران

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چکیده

صد و پنجاه نمونه مشکوک به کلی باسیلوز از جوجه های گوشتی جدا شد. مرغ های تخمگذار و مرغ مادر از ۲۱ مزرعه نمونه برداری شدند. آزمایش در ۹۸ مورد مثبت بود. و حساسیت آنها به ده نوع آنتی بیوتیک با استفاده از روش آنتی بیوگرام، روش انتشار دیسک و قطر هاله مهار رشد بر حسب میلی متر بررسی شد. ۹۰ مورد مثبت (۶۰٪) مرغ گوشتی و ۸ مورد مثبت (۳۳٪) مرغ مادر و تخمگذار بودند. دیسک های آنتی بیوتیک مربوط به تتراسایکلین، آمپی سیلین، دیفلوکساسین، انروفلوکساسین، داکسی سایکلین، سولفامتو کسازول + تری متوپریم، سیپروفلوکساسین، کلستین، فلورفنیکول، فوزبک بود. بیشترین هاله مربوط به فلورفنیکال و فوزبک (۲۱-۲۲ میلی متر) بود. همچنین کوچکترین هاله مربوط به تتراسایکلین و آمپی سیلین (۸-۹ میلی متر) بود. میانگین قطر هاله در بین ده آنتی بیوتیک از کمتر از ۸ میلی متر تا بیش از ۲۲ میلی متر اندازه گیری شد. همه اشریشیا کلی های جدا شده حداقل ۲۶ درصد به ۷ نوع آنتی بیوتیک مقاوم بودند. آنتی بیوتیک های نسل قدیم مانند تتراسایکلین و آمپی سیلین نسبت به آنتی بیوتیک های نسل جدید تأثیر کمتری داشتند. در نتیجه، فراوانی مقاومت آنتی باکتریایی به دلیل مصرف بی رویه آن ها، بالا بود و می تواند خطری جدی برای صنعت طیور کشور و سلامت عمومی به شمار رود.

واژه های کلیدی: کلی باسیلوز، آنتی بیوتیک، اشریشیا کلی، طیور

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INTRODUCTION

Escherichia coli is a type of gram-negative bacillus of the Enterobacteriace family which causes colibacillosis in poultry. *Escherichia coli* bacterial syndrome in poultry has the signs such as air sac swelling, umbilical edema, septicemia, intestinal infection, oviduct infection, granuloma coli, joint swelling, panophthalmia and pericarditis [1]. Certain strains of this bacterium can cause various diseases under certain conditions. Most *Escherichia coli* strains are harmless, but some serotypes such as O157:H7 cause food poisoning and diarrhea in humans. Harmless strains of these bacteria are part of the natural flora of the intestine. This bacterium constitutes one percent of the human intestinal flora and is transmitted from person to person through the fecal-oral route [2]. Colibacillosis causes great economic damage to the poultry industry every year [6]. This disease is observed in chickens, ducks and turkeys and the mortality rate is less than 5% while the infection rate reaches 50%. Poultry gets this disease at the age of 4 to 12 weeks. Infected chickens are lethargic and lose food and water [3]. The bacterium spreads to other chickens and leads to yolk sac infection in chickens [4]. With the emphasis on the increase of antibiotic resistance and the reduction of biotics isolated from *Escherichia coli*, which is usually due to the consumption of antibiotics in poultry [5], Antibiotics resistance investigation is needed for this case. Therefore, the present study was conducted on poultry infected with *Escherichia coli* in Kohgiluyeh and Boyer-Ahmad province of Iran.

MATERIALS AND METHODS

One hundred and fifty samples were selected from a total of 21 industrial units of breeding

broilers, mothers and laying hens suspected to colibacillosis disease in Kohgiluyeh and Boyer-Ahmad province.

The samples were taken from the poultry which was suspected to general colibacillosis with clinical symptoms and mortality. The samples were immediately cultured in sterile conditions and by Eosin-Methylene Blue Agar (EMB) transfer medium sent to Yasooj microbiology laboratory. *Escherichia coli* in EMB environment produce metallic green polish. Ninety total cases of colibacillosis (60%) were related to meat poultry and 8 cases (5.33%) were related to mother and egg-laying birds (Table 1). Out of 150 suspected cases of colibacillosis, 98 cases were positive for *Escherichia coli* during biochemical tests and EMB's special culture medium. Antibiogram tests were performed with some antibiotics and their lack of growth was measured.

RESULTS

The antibiotic discs that were used, related to tetracycline, ampicillin, difloxacin, enrofloxacin, doxycycline, sulfamethoxazole + trimethoprim, ciprofloxacin, calcitin, florfenicol, Fosbak. The biggest halo was florfenicol and Fosbak (21-22 mm). And the smallest halo is tetracycline and ampicillin (8-9 mm). There was an average halo diameter among ten antibiotics. It was measured from less than 8 mm to more than 22 mm. All isolated *Escherichia coli* were at least 26% resistant to 7 types of antibiotics. Old generation antibiotics such as tetracycline and ampicillin were less effect than new generation antibiotics. In the present study, by referring to 21 broiler and mother hen breeding centers in Kohgiluyeh and Boyer-Ahmed province, 98 cases of *Escherichia coli* were positive out of 150 suspected cases colibacillosis in the

special culture medium and biochemical test, and 52 cases were infected with salmonella, of which 90 total cases of bacillus (60%) were related to meat-rearing poultry and 8 cases (33.5%) were related to mother and egg-laying birds (Table 1). According to the statistical analysis, colibacillosis was observed more frequently (up to 60%) in broiler chickens at the age of 20-40 days. It was also observed

antibiotic resistance than the three newer antibiotics (8-10) (Table 2).

DISCUSSION

There was no difference in the pattern and level of drug resistance of poultry pathogen *Escherichia coli* to antibacterial drugs. As in

Table 1. Percentage of infection by *Escherichia coli* in Leghorn breeds broilers between 20 and 60 days old

poultry	Number of patients	Percentage of infection
Broiler chickens (1-60 days) with colibacillosis	90	60
Mother hens and laying hens	8	5.33
Generalized bacillosis		
Total cases of colibacillosis	98	65.33

Table 2. Antibiotic susceptibility testing (AST) for *Escherichia coli* in this study

Antibiotic	Number of Discs	Average Drops inhibit <i>Escherichia coli</i> Growth	Percentage of <i>Escherichia coli</i> Resistance
Tetracycline	10	8	64
Ampicillin	10	9	55
Florfenicol (chloramphenicol family)	10	21	7
Fosbak (phosphomycin 25% and fructose)	8	22	5

The greatest growth inhibition halo is related to the florfenicol and Fosbak (22-21 mm). The lowest growth inhibition zone is tetracycline and ampicillin (8-9 mm). The average diameter of growth inhibition zone among the ten antibiotics used was measured from less than 8 to more than 22 mm.

more frequently in the Leghorn chicken breed. We concluded that the general disease of colibacillosis had a lot to do with the age and race factors. The results of the biochemical diagnosis of (IMVIC) test were the same for all 98 isolated *E. coli* cases that were subjected to microbial differential diagnosis. All isolated *Escherichia coli* had resistance to at least 7 antibiotics above 26%. As can be seen, the older generation antibiotics (1-7) showed more

other studies, drug resistance in *Escherichia coli*, was a serious and necessary issue in the poultry industry. In the present study we also observed drug resistance in isolated *Escherichia coli*. Haghghi et al (2010), investigated the antibacterial resistance patterns of *Escherichia coli* isolated from broiler chickens infected with *Escherichia coli* in a study in the north of Iran, Golestan province at the level of broiler farms. The

results of this study indicated that all *Escherichia coli* isolates were resistant to nalidixic acid, erythromycin, flumequine and sensitive to gentamicin and ceftiofur. More than 80% of *Escherichia coli* isolates were resistant to ciprofloxacin, tetracycline, sulfadiazine + trimethoprim, enrofloxacin, ciprofloxacin, danofloxacin, lincospectin, difloxacin, sulfachloropyridazine + trimethoprim, and doxycycline. Also, the frequency of *Escherichia coli* tested in this study for antibiotic compounds showed that there are 29 patterns and 88% of *Escherichia coli* isolated in that study belonged to more than one pattern and 12% of them each belonged to only one pattern [8, 10]. In two studies that were conducted on drug resistance against *Escherichia coli* in Shiraz and of Tehran provinces in year 2004, it was found that the resistance of the isolates to antibacterial compounds was wide and high [9]. In a study was conducted by Jahantigh et al. in Zabol city on 100 broiler chickens infected with colibacillosis to determine the distribution of phylogenetic groups, they demonstrated that from the total of 100 isolates, 36%, 27%, 23% and 14% were placed in the phylogenetic groups A, D, B1, B2, respectively, and it was determined that most of the *Escherichia coli* isolated from broiler chickens with colibacillosis in Zabol city belonged to the B1 phylogenetic group [11]. In another study that was conducted on 150 samples of egg-laying and broiler chickens infected with colibacillosis to determine phylogenetic groups, they observed that 8.31%, 7.21%, 2.21% and 25.3% of isolates belonged to B2, B1, A and D groups. The majority of the isolates belonged to the phylogenetic group A and then group D were the dominant isolates among the isolates obtained from the general cases of broiler and egg-laying bacillus [4]. Moreover, Amara et al., declared the difference in the pattern and level of *Escherichia coli* drug resistance of the

poultry pathogen to antibacterial drugs could be seen. The pattern of drug resistance against *Escherichia coli* in Canada as well as in Tehran province of Iran have been reported [3]. In another study in 2018 entitled "Comparison of the effect of antibiotics on *Escherichia coli* isolated from chicken farms in Qazvin province", the effect of different antibiotics was investigated, and the results obtained in that study indicated that 48% of the checked serotypes are pathogenic for 3-week-old chickens and 10-15% of intestinal coliforms of birds [12]. It belonged to pathogenic serotypes, and the resistance level of *Escherichia coli* isolates to antibacterial compounds was wide and high [4]. In studies conducted in different parts of the world, the situation of antibiotic resistance in different birds has been differently. Thai is, in some studies, a very high rate of antibiotic resistance has been reported, while in others, a lower prevalence has been reported. Dolejska et al (2007) in a study they conducted on *E. coli* isolated from black-headed chickens announced that 29.9% of the *Escherichia coli* species isolated were resistant to antibiotics. All the strains that developed resistance were resistant to at least one or more antibiotics. Among the tested antibiotics, there was the highest resistance to tetracycline (19.1%) and the lowest resistance to chloramphenicol (1.9%). In another study conducted in northeastern Czechoslovakia, 28% of *E. coli* isolated from Nowruz chickens were resistant to antibiotics. Among the tested antibiotics, there was the highest resistance to tetracycline (22%), but no resistance was seen to ceftriaxone and ceftazidime [7]. In a study conducted in France on the transmission of beta-lactamase-producing *E. coli* between humans and yellow-claw chickens, it was announced that 47.1% of the isolates were resistant to at least one or more antibiotics. There was the highest resistance to tetracycline

and the lowest resistance to nalidixic acid and chloramphenicol [6]. The process of indiscriminate and incorrect use of antibiotics in poultry breeding units causes diversity in the pattern of drug resistance in that area, and this probably leads to an increase in genes resistant to antibacterial compounds among pathogenic *Escherichia coli* and naturally the phenomenon of antibiotic resistance selection pressure will follow [6].

CONCLUSION

This study and similar studies show that antibiotic resistance patterns are different in different regions and resistance to antibiotics is an issue that should be taken seriously. Considering the increase in the prevalence of resistance to antibiotics, quick and timely detection of resistant strains in order to choose appropriate treatment options and prevent the spread of resistance seems essential. The results of this study are a re-emphasis on the results of the sensitivity tests of bacteria against antimicrobial agents in order to prevent the increase of drug resistance by choosing the appropriate antibiotics in addition to effective treatment. Considering the high amount of antibiotic resistance in strains, compared to the results obtained from similar research in recent years, it seems that the phenomenon of drug resistance in developing countries is increasing at an increasing rate, and this statistics and figures show that the city of Yasouj is facing this phenomenon in a very large and negligible amount. Careful measures by clinical practitioners to prescribe the appropriate antibiotic and control the general disease of colibacillosis and prevent the spread of drug resistance (the use of appropriate antibiotics will prevent the transmission of resistance by integron) should be at the top of the list. Also, accurate determination of genotypic factors of

resistance to better deal with these factors should be on the agenda of future research.

Suggestions: Preventing the spread of drug resistance is one of the most important issues in the treatment of infections in society. Due to the increase in the prevalence of resistance to antibiotics, today we see numerous reports on their widespread prevalence in different parts of poultry farms. Therefore, quick and timely detection of resistant strains in order to choose appropriate treatment options and prevent the spread of resistance seems essential. It is also suggested that the treatment is of particular importance, taking into account the pattern of sensitivity and resistance of the region, in order to avoid the phenomenon of drug resistance and treatment failures that lead to complications of the infection.

ETHICS

Approved.

CONFLICT OF INTEREST

None declared.

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