Islamic Azad University
Urmia Branch

Journal of Basic and Clinical Veterinary Medicine

2022; 3(1): 1-6

Official Journal of Veterinary Faculty of Islamic Azad University
Urmia Branch

Journal Homepage: jbcvm.iaurmia.ac.ir

Original Article

Evaluation of antibiotic resistant and molecular identification of *Escherichia coli* isolates from poultry colibacillosis in Tabriz city during year 2020

Saman Mahdavi

Department of Microbiology, Maragheh Branch, Islamic Azad University, Maragheh, Iran

ARTICLE INFO

Received: 4 October 2021

Accepted: 12 September 2022

DOI:10.30495/jbcvm.2022.1941910.1019

KEYWORDS:

Colibacillosis
Escherichia coli
Antibiotic resistance
Poultry

ABSTRACT

This study conducted to evaluation of antibiotic resistant and molecular identification of *Escherichia coli* isolates from poultry colibacillosis in Tabriz city during 2020. Forty-six *Escherichia coli* samples from colibacillosis cases of 10 aviculture in Tabriz city, which had been previously isolated and detected by microbiological and biochemical methods, were identified molecularly with 16s rRNA primers by PCR method. To determine the pattern of antibiotic resistance of isolates, common antibiotics available in the market were used by Kirby-Bauer method. Of 46 *Escherichia coli* samples tested, 40 isolates (86.9%) were identified as *Escherichia coli*. All strains of *Escherichia coli* (100%) were resistant to ampicillin. The most strains were resistant to ceftriaxone (75%), neomycin (70%) and nitrofurantoin (70%). Twelve *Escherichia coli* strains (30%) were resistant to all of tested antibiotics. It can be concluded that the antibiotic resistance of *Escherichia coli* isolates to common antimicrobial drugs used in the poultry industry is high. So, implementation of national monitoring programs is strongly needed for antimicrobial resistance and rational use of antibiotics.

بررسی مقاومت آنتی‌بیوتیکی و شناسایی مولکولی جدایه‌های *اشریشیا کلی* از موارد کلی‌باسیلوز طیور در شهر تبریز در سال ۱۳۹۹

سامان مهدوی

گروه میکروبیولوژی، واحدمراغه، دانشگاه آزاد اسلامی، مراغه، ایران

چکیده

تحقیق حاضر با هدف بررسی مقاومت آنتی‌بیوتیکی و شناسایی مولکولی جدایه‌های *اشریشیا کلی* از موارد کلی‌باسیلوز طیور در شهر تبریز در سال ۱۳۹۹ انجام گرفت. ۴۶ نمونه *اشریشیا کلی* از موارد کلی‌باسیلوز ۱۰ مرغداری شهر تبریز که با روش‌های میکروب‌شناسی و بیوشیمیایی جداسازی و تشخیص داده شده بودند، جهت شناسایی مولکولی با پرایمرهای ژن 16s rRNA، PCR شدند. جهت تعیین الگوی مقاومت آنتی‌بیوتیکی جدایه‌ها، از آنتی‌بیوتیک‌های رایج موجود در بازار به روش کربی بائر استفاده شد. از ۴۶ نمونه *اشریشیا کلی* مورد آزمایش، ۴۰ جدایه (۸۶/۹٪) به عنوان باکتری *اشریشیا کلی* مورد تایید قرار گرفت. تمام جدایه‌های مورد آزمایش (۱۰۰٪)، در برابر آنتی‌بیوتیک آمپی‌سیلین از خود مقاومت نشان دادند. اکثر جدایه‌های *اشریشیا کلی* در برابر سفتریاکسون (۷۵٪)، نئومایسین (۷۰٪) و نیتروفورانتوئین (۷۰٪) از خود مقاومت نشان دادند. ۱۲ جدایه *اشریشیا کلی* (۳۰٪)، در برابر تمام آنتی‌بیوتیکهای مورد آزمایش مقاوم بودند. می‌توان نتیجه‌گیری نمود که مقاومت آنتی‌بیوتیکی جدایه‌های اشریشیا کلی نسبت به داروهای ضد میکروب متداول مورد مصرف در صنعت پرورش طیور بالا می‌باشد که اجرای طرح پایش ملی برای مقاومت ضد میکروبی و مصرف اصولی آنتی‌بیوتیک ها ضروری به نظر می‌رسد.

واژه های کلیدی: کلی‌باسیلوز، *اشریشیا کلی*، مقاومت آنتی‌بیوتیکی، طیور

* Corresponding author: S.mahdavi@iau-maragheh.ac.ir

INTRODUCTION

Avian pathogenic *Escherichia coli* (APEC) causes localized or systemic infection outside the avian gut, which indicates as Extraintestinal Pathogenic *E. coli* (ExPEC). The infection caused by ExPEC is termed colibacillosis which is an infectious disease characterized by acute fatal septicemia or sub-acute fibrinous pericarditis, airsacculitis, salpingitis, and peritonitis affect broiler chickens aged 4–6 weeks [1, 2]. Colibacillosis is a common bacterial disease of economic importance in poultry through decreasing the infected birds' productivity, increase mortality, condemnation of infected carcasses at slaughter, and prophylaxis and treatment cost and is reported worldwide [2]. APEC is considered a primary or secondary pathogen of poultry. Strains which carry virulence genes (adhesin, invasins, toxins, resistance to host serum, iron acquisition systems, temperature-sensitive hemagglutinin, and K1 capsule), have been shown to contribute to APEC pathogenesis [3, 4] and could induce colibacillosis without previous immune suppression factors; stress or concurrent infections [5]. *Escherichia coli* infection is of particular importance and imposes significant economic losses on the poultry industry annually in the world due to the increase in losses and the increase in carcass condemnation in the slaughterhouse inspection process [6]. In order to control the complications of the disease and mortality due to *Escherichia coli* infection, many antimicrobial agents are used in the poultry industry [7]. However, in recent years, the increasing use of drugs as a prevention and treatment of infections or to enhance the growth of poultry has led to the emergence and spread of antibiotic resistant genes. Therefore, it increases the antibiotic resistance of bacteria, which has reduced the effectiveness of drugs and as a result, the treatment of this disease has become difficult. On the other hand, the phenomenon of antibiotic resistance is of special importance for the spread of resistant bacteria in human communities in terms of public health [8]. The objectives of the current study were to evaluation of antibiotic resistant and molecular identification of *Escherichia coli* isolates from poultry colibacillosis in Tabriz city during year 2020.

MATERIALS AND METHODS

Sample collection

Forty-six samples of *Escherichia coli* isolated from poultry colibacillosis from 10 poultry farms (from April to October in 2020) located in Tabriz city (East Azarbaijan province, Iran) that had been previously identified by various microbiological and biochemical tests, considered in this study for molecular tests and antibiotic resistance pattern.

DNA extraction

DNA extraction of *Escherichia coli* strains was performed in brain heart infusion (BHI) (Merck company, Germany) medium by boiling method [9]. The quality and quantity of extracted DNA were evaluated by electrophoresis on 1.5% agarose gel and nanodrop device.

Molecular detection of Escherichia coli

To confirm the phenotypic diagnosis of *Escherichia coli* bacteria isolated from poultry colibacillosis, genotypic diagnosis of isolates was performed using genus specific primer by PCR method. The polymerase chain reaction (PCR) method was done in 25 µl, including 11 µl of Master mix PCR, 1 µl of each specific

primers (25 nano moles) (Table 1), 1 µl (50 ng) of DNA template and 11 µl of double distilled water. Proliferation of intended gene in thermocycler was performed as following: primary denaturation at 94°C for 4 min, 35 cycles with denaturation stage at 94°C for 30 s, annealing stage at 55°C for one min, extension stage at 72°C for one min, and finally, a terminal extension stage at 72°C for 5 min. The amplified products were run on 1.5% agarose gel and staining with ethidium bromide (0.5 mg/ml) in a dark room [10].

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing of all identified isolates were done according to the criteria of the Clinical and Laboratory Standards Institute method [11] (Padtan teb, Iran) (Kirby-Bauer method). Antibiotic discs were used as follows: ampicillin (10 µg), ceftriaxone (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), cotrimoxazole (23.75 µg), neomycin (30 µg), nitrofurantoin (300 µg), tetracycline (30 µg).

RESULTS

Of 46 *Escherichia coli* tested samples, 40 isolates (86.9%) were identified as *Escherichia coli* by complementary molecular tests with 16s rRNA specific primer (Figure 1). The antimicrobial susceptibility test results (Table 2) showed that, all samples of *Escherichia coli* (100%) were resistant to ampicillin. Also, most samples were resistant to ceftriaxone (75%), neomycin (70%) and nitrofurantoin (70%). The highest susceptibility of *Escherichia coli* samples was to chloramphenicol (47.5%) and ciprofloxacin (40%). The antibiogram results showed that 12 samples of *E. coli* (30%) were resistant to all of tested antibiotics (Table 3).

Table 1. Characteristics of specific primers related to the gene under investigation

Gene	primer sequence (5'→3')	(bp) Amplicon size	Reference
16srRNA	F- AGAGTTTGATCMTGGCTCA R- CCGTCAATTCATTTGAGTTT	919	10

Figure 1: Lane 1 is marker (100 bp). Lane 2 is positive control (*Escherichia coli* PTCC 1163) that bands within 919 bp. Lane 3 is negative control (Double distilled water). Lanes 4, 5, 8, 10, 12 and 13 are positive samples. Lanes 6, 7, 9, 11, 14 and 15 are negative samples.

DISCUSSION

Colibacillosis is economically one of the most important bacterial diseases of the poultry industry, especially broilers in the world [12]. Today, the fight against this disease relies more on the use of antimicrobial compounds. Extensive and long-term administration of antibiotics has led to resistance to some species of bacteria, and this resistance has ultimately led to ineffective drugs and treatment failure. In recent decades, according to several reports, the resistance of pathogenic *Escherichia coli* strains of poultry to antimicrobial compounds used in the poultry industry has been increasing and the pattern of antibiotic resistance in different geographical areas is diverse and changing [13, 14]. According to the results of this study, all strains of *Escherichia coli* (100%) were resistant to ampicillin. Most of the samples were resistant to ceftriaxone (75%), neomycin (70%) and nitrofurantoin (70%). The highest sensitivity of *Escherichia coli* strains was to chloramphenicol (47.5%) and ciprofloxacin (40%). Azizpour and Saeidi Namin (2017) in Ardebil province reported that the resistance of *Escherichia coli* isolated strains from poultry colibacillosis to tetracycline, ampicillin and neomycin was 99.43%, 60.11% and 75.84%, respectively [15]. Gregova et al (2012) in Slovakia showed that the resistance of *Escherichia coli* isolated strains from poultry slaughterhouse to tetracycline and ampicillin was 33% and 89%, respectively [16]. Mohammadi (2018) reported that the resistance to neomycin and ampicillin in *E. coli* isolated samples from poultry in Kermanshah city was 13% and 27%, respectively [17]. The reason for the discrepancy between the results of the antibiogram test in several studies is probably due to differences in geographical area, type of treatment and the amount of antibiotic use in different regions [18]. Bacterial antimicrobial resistance develops naturally over time. The unique increase of antimicrobial resistant organisms is related to the massive use of antimicrobial agents for disease control and

Table 2. Antibiogram results of *Escherichia coli* samples isolated from poultry colibacillosis

Antibiotic	Abbreviation	Concentration (µg)	Susceptibility		
			R (%)	S (%)	I (%)
Ampicillin	AM	10	40 (100)	0 (0)	0 (0)
Ceftriaxone	CRO	30	30 (75)	3 (7.5)	7 (17.5)
Chloramphenicol	C	30	18 (45)	19 (47.5)	3 (7.5)
Ciprofloxacin	CP	5	18 (45)	16 (40)	6 (15)
Cotrimoxazole	SXT	23.75	25 (62.5)	13 (32.5)	2 (5)
Neomycin	N	30	9 (22.5)	28 (70)	3 (7.5)
Nitrofurantoin	FM	300	7 (17.5)	28 (70)	5 (12.5)
Tetracycline	TE	30	7 (17.5)	11 (27.5)	22 (55)

Note: R: Resistant, S: Sensitive, I: Intermediate

Table 3. Frequency and percentage distribution of multidrug resistant (MDR) *Escherichia coli* isolates

Resistant isolates (%)	1 fold*	2 fold	3 fold	4 fold	5 fold	6 fold	7 fold	8 fold
	40 (100)	39 (97.5)	38 (95)	35 (87.5)	33 (82.5)	28 (70)	22 (55)	12 (30)

*= fold resistance

prevention in human and animal medicine [19]. The frequent use of antimicrobial agents give rise to selective pressure that lead to antimicrobial resistance against APEC. The development of resistance is a complex process associated with the presence of resistance encoding genes that found inside plasmids or chromosomal genetic material [20]. Antimicrobial resistant *E. coli* strains create a serious public health problem, because they can be transmitted to humans through the food chain or in direct contact with infected birds. In addition, resistant *E. coli* may act as vectors for antimicrobial resistant genes to other pathogens [21]. In many developed countries, the administration of antimicrobial agents is not limited to therapeutic purposes only. Antimicrobials can also be used to increase animal productivity, feed conversion rate, and growth rate in food-producing animals [22]. This type of farming practice allows antimicrobial drugs to eliminate sensitive bacterial strains and select strains with genetic characteristics that can resist antimicrobials, which provides favourable conditions for selected strain to survive and distribute at the field level [23]. The use of antimicrobial agents as feed additives, administered at low concentrations (sub-therapeutic dose) usually over long periods of time, may lead to development of resistance [24, 25]. With the growing trend of antibiotic resistance, the principles of proper nutrition and hygiene and the use of appropriate management practices such as selecting healthy chickens, proper nutrition, improving the litter, exterminating infected chickens, preventing disease from entering the farm and timely vaccination, should be observed. Therefore, both the incidence of infectious diseases in poultry and the use of various antibiotics, will be greatly reduced.

CONCLUSION

The results of current study showed high frequency of antibiotic resistance in *Escherichia coli* isolated samples from poultry colibacillosis. It may occur due to the exchange of resistance genes within and between species and with common bacteria in humans and animals. By examining the diversity and frequency of drug resistance patterns of isolates in this area, it is possible to obtain accurate information about the selection of appropriate and effective antibiotics for the treatment of colibacillosis.

ETHICS

All ethical standards have been respected in this study.

CONFLICT OF INTEREST

None declared.

REFERENCES

[1] Alexander DJ, Senne DA. Newcastle disease, other avian paramyxoviruses, and Pneumovirus infections. Disseases of poultry; 2008.

[2] Lutful Kabir SM. Avian colibacillosis and salmonellosis: a closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. International Journal of Environmental Research and Public Health. 2010; 7(1):89–114.

[3] Dziva F, Stevens MP. Colibacillosis in poultry: unravelling the molecular basis of virulence of avian pathogenic *Escherichia coli* in their natural hosts. Avian Pathology. 2008; 37(4):355–66.

[4] Mellata M, Dho-Moulin M, Dozois CM, Curtiss R, Brown PK, Arne P, et al. Role of virulence factors in resistance of avian pathogenic *Escherichia coli* to serum and in pathogenicity. Infection and Immunity. 2003;71(1): 536–40.

[5] Collingwood C, Kemmett K, Williams N, Wigley P. Is the concept of avian pathogenic *Escherichia coli* as a single pathotype fundamentally flawed? Frontiers in Veterinary Science. 2014;1: 1–4.

[6] Peighambari SM, Vaillancourt JP, Wilson RA, Gyles CL. Characteristics of *Escherichia coli* isolates from avian cellulitis. Avian Diseases. 1995;65: 116- 124.

[7] Nolan LK, Barnes HJ, Vaillancourt JP, Abdul-Aziz T, Logue CM. colibacillosis. In: diseases of poultry. 12th edition. (Swayne DE, Mcdougald L, Nolan K, Suarez DL, Nair V). Iowa, USA: John Wiley and Sons, 2013.

[8] Johnson JR, Kuskowski MA, Smith K, O-Bryan TT, Tatini S. Antimicrobial-resistant and extra intestinal pathogenic *Escherichia coli* in retail foods. Journal of Infectious Diseases. 2005;191: 1040-9.

[9] Chen J, Su Z, Liu Y, Wang S, Dai X, Li Y, et al. Identification and characterization of class 1 integrons among *Pseudomonas aeruginosa* isolates from patients in Zhenjiang, China. International Journal of Infectious Disease. 2009; 13:717-721.

[10] Goudarzi V, Mirzaee M, Ranjbar R. O-serogrouping of *Escherichia coli* strains isolated from patients with urinary tract infection by using PCR method. Iranian Journal of Medical Microbiology. 2017; 10(6): 1-8.

[11] CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI supplements M100. Wayne, PA: Clinical and Laboratory Standards Institute, 2017.

[12] Nolan LK, Barnes HJ, Vaillancourt JP, Abdul-Aziz T, Logue CM. Colibacillosis. In: diseases of poultry. 12th edition. (Swayne DE, Mc dougald L, Nolan K, Suarez DL, Nair V). Iowa, USA: John Wiley and Sons, 2013.

[13] Hanson R, Kaneene JB, Padungtod P, Hirokawa K, Zeno C. Prevalence of salmonella and *E. coli* and their resistance to antimicrobial agents, in farming communities in northern Thailand. Southeast Asian Journal of Tropical Medicine and Public Health. 2002;33: 120-126.

[14] Ozawa M, Harada K, Kojima A, Asi T, Sameshima T. Antimicrobial susceptibilities, serogroups, and molecular characterization of avian pathogenic *Escherichia coli* isolates in Japan. Avian Diseases. 2008; 52: 392-397.

[15] Azizpour A, Saeidi Namin V. Investigation of antibiotic resistance patterns in *Escherichia coli* isolated from broiler chickens with colibacillosis to ten antibacterial agents commonly used in the Iranian poultry industry. Journal of Comparative Pathobiology. 2017;14(4): 2345-2352.

[16] Gregova G, Kmetova M, Kmet V, Venglovsky J, Feher A. Antibiotic resistance of *Escherichia coli* isolated from a poultry slaughterhouse. Annals of Agricultural and Environmental Medicine. 2012;19(1): 75-77.

[17] Mohammadi F. Antibiotic resistance determination in *Escherichia coli* isolated from poultry samples enffected colibacillosis disease from around Kermanshah farms. Journal of Food Microbiology. 2018; 5(2): 72-78.

[18] Enferad E, Mahdavi S. Antibiotic resistance pattern and frequency of some beta lactamase genes in *Klebsiella pneumoniae* isolated from raw milk samples in Iran. Journal of the Hellenic Veterinary Medical Society. 2021;71(4), 2455-2462.

[19] Levy S. Reduced antibiotic use in livestock: how Denmark tackled resistance. Environ Health perspect. 2014; 122(6):160-5.

[20] Awad A, Arafat N, Elhadidy M. Genetic elements associated with antimicrobial resistance among avian pathogenic *Escherichia coli*. Annals of Clinical Microbiology and Antimicrobials. 2016; 15(1):59.

[21] Akond M. Antibiotic resistance of *Escherichia coli* isolated from poultry and poultry environment of Bangladesh. American Journal of Environmental Sciences. 2009;5(1):47–52.

[22] Snary EL, Kelly LA, Davison HC, Teale CJ, Wooldridge M. Antimicrobial resistance: a microbial risk assessment perspective. Journal of Antimicrobial Chemotherapy, 2004;53(6):906–17.

[23] Castanon JIR. History of the use of antibiotic as growth promoters in European poultry feeds. Poultry Sciences. 2007;86(11):2466–71.

[24] Diarra MS, Malouin F. Antibiotics in Canadian poultry productions and anticipated alternatives. Frontiers in Microbiology. 2014; 5:1–15.

[25] Apata DF. Antibiotic resistance in poultry. International Journal of Poultry Science. 2009;8(4):404–8.