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RESEARCH ARTICLE

Distribution of Serotypes and Antibiotic Resistance of Avian Pathogenic *Escherichia coli* Strains Isolated from Chickens

Nurdan KARACAN SEVER [1 (*)] Özlem ŞAHAN YAPICIER [2] Mehmet AKAN [3]

[1] Dicle University, Faculty of Veterinary Medicine, Department of Microbiology, TR-21200 Diyarbakır - TÜRKİYE

[2] Republic of Turkey Ministry of Agriculture and Forestry Veterinary Control, Central Research Institute, Bacteriology Diagnostic Laboratory, TR-06020 Ankara - TÜRKİYE

[3] Ankara University, Faculty of Veterinary Medicine, Department of Microbiology, TR-06110 Ankara - TÜRKİYE

ORCIDs: N.K.S. 0000-0002-0618-5822; Ö.Ş.Y. 0000-0003-3579-9425; M.A. 0000-0002-7342-1450

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Abstract: Avian pathogenic *Escherichia coli* (APEC) causes systemic or localized infections with different clinical courses such as septicemia, air sac disease, polyserositis and coligranuloma. Colibacillosis causes significant economic losses in the poultry industry due to the need for the control of the disease by causing carcass contamination, decreased feed conversion rate, mortality in poultry. In this study, 99 *E. coli* strains were isolated from different production units in the same integration. Antimicrobial susceptibility tests of all strains were performed and thirty strains with multidrug resistance (MDR) in the different production units were serotyped. The highest resistance was observed against oxytetracycline, erythromycin amoxicillin and doxycycline. Multidrug resistance was observed at a rate of 81.81% (81/99). Among the 30 strains that could be serotyped, O78 and O125 were determined as the most prevalent serogroups with 43.3% (13/30) and 16.6% (5/30) rates, respectively, while O1, O8, O18, O142, O143, O157, O158, O164 and O169 were found as rare serogroups. O78 was determined as a high antibiotic resistant strain in isolated *E. coli* strains and a dominant serotype in the selected strains and this study demonstrated that a correlation can be between breeders and their progeny.

Keywords: *Antibiotic resistance, Chicken, Escherichia coli, Serogroups*

Tavuklardan İzole Edilen Avian Patojenik *Escherichia coli* Suşlarının Antibiyotik Direnci ve Serotip Dağılımı

Öz: Avian patojenik *Escherichia coli* (APEC) koligranuloma, poliserözitis, hava kesesi yangısı, septisemi gibi farklı klinik seyirler izleyerek sistemik ya da lokal enfeksiyonlara sebep olmaktadır. Kolibasillozis kanatlı hayvanlarda karkas kontaminasyonu, yem dönüşümünde azalma, mortaliteye sebep olabildiğinden hastalığın kontrolü, kanatlı hayvan endüstrisinde önemli ekonomik kayba neden olmaktadır. Bu çalışmada aynı entegrasyona ait farklı üretim birimlerindeki kanatlı hayvanlardan 99 *E. coli* suşu izole edildi. Tüm suşların antimikrobiyal duyarlılıkları test edildi ve farklı üretim birimlerindeki çoklu ilaç direncine sahip 30 suş serotiplendirildi. En yüksek direnç oksitetrasiklin, eritromisin, amoksisilin ve doksisiline karşı belirlenmiştir. %81.81 (81/99) oranında çoklu ilaç direnci gözlenmiştir. Serotiplendirilen 30 suş arasında O1, O8, O18, O142, O143, O157, O158, O164 ve O169 ise nadir serogruplar olarak belirlenirken O78 %43.3 (13/30) ve O125 %16.6 (5/30) en yaygın serogruplar olarak saptanmıştır. İzole edilen *E. coli* suşlarında yüksek antibiyotik direnci ve serotiplendirilen *E. coli* suşlarında O78'in baskın serotip olduğu tespit edildi. Ayrıca bu çalışma damızlık ve bu damızlıklara ait soylardan izole edilen *E. coli* suşları arasında bir ilişki olabileceğini de ortaya koymuştur.

Anahtar sözcükler: *Antibiyotik direnci, Tavuk, Escherichia coli, Serogrup*

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(*) Corresponding Author

Phone: +90 412 241 1000/8699 Cellular phone: +90 544 739 9628
E-mail: nurdankaracan@hotmail.com (N. Karacan Sever)

Introduction

Escherichia coli is an important member of the gut microbiota of humans and animals. However, some strains are potentially pathogenic and cause both enteric and extraintestinal infections in humans and animals [1,2]. APEC is a subgroup of extraintestinal pathogenic *E. coli* (ExPEC), a pathotype that also includes uropathogenic *E. coli* (UPEC) and neonatal meningitis *E. coli* (NMEC) of human [2-4]. APEC, called avian colibacillosis in chickens, turkeys, and other avian species, causes various clinical syndromes including colisepticemia, coligranuloma (Hjarre's disease), air sac disease (chronic respiratory disease, CRD), swollen-head syndrome, peritonitis, yolk sac infection, polyserositis, pericarditis, perihepatitis, salpingitis and enteritis [5-7]. APEC infections have significant morbidity and mortality in birds of all ages, resulted with serious economic losses in the poultry industry [6,7]. Diseases such as mycoplasmosis, Newcastle disease, infectious bursal disease, coccidiosis or nutritional deficiency etc. predispose all poultry to colibacillosis [5,7]. Reducing the incidence and mortality and successful treatment of avian colibacillosis mainly depends on the use of antibiotics [4,6,8]. However, misuse and overuse of antibiotics have led to the development of antibiotic resistance. Contamination of poultry carcass by antibiotic resistant bacteria may increase the risk of transmission of antibiotic resistance genes to humans [4,8,9]. The World Health Organization (WHO) has published a list of antibiotic-resistant "priority pathogens" of 12 families of bacteria of international concern. *E. coli* has been included in the list of 12 microorganisms of international importance that cause the most prevalent infections in different areas [10].

APECs are characterized by many methods, including serotyping. The most common serotypes associated with infections of APECs isolated from septicemic chickens studied to date have been shown to be O1, O2, O5, O8, O11, O15, O25, O78, and O88. Other serotypes were found less frequently. Their prevalence varies depending on many factors such as the region of origin of the disease, age, species and utility type of the poultry [3,11-16]. There are a few studies regarding to avian *E. coli* and antibiotic resistance in our country [17-19].

The aim of this study is to determine antibiotic resistance in APEC strains isolated from different poultry production units (broiler, breeding, hatchability) and common serotypes in multidrug resistant APEC strains.

Material and Methods

Sampling and Cultural Examination

Avian *E. coli* strains were isolated from internal organs (pericardial fluid, heart blood, liver and spleen) collected from 21 broilers, 35 breeding flocks that were diagnosed with colibacillosis and 33 eggs from the incubator during hatchery were diagnosed one-day chick dead in the same integration in Türkiye. Samples were cultured on MacConkey agar and incubated overnight at 37°C aerobically. Lactose positive, pink colonies were selected and identified as *E. coli* by biochemical tests [6]. All strains were kept at -20°C in Luria-Bertani (LB) medium containing 15% glycerol until tests were performed.

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility of all *E. coli* strains was determined using Kirby-Bauer disc diffusion method according to the standards Clinical and Laboratory Standards Institute (CLSI) [20]. Each strain was tested for susceptibility to florfenicol (30 µg), erythromycin (15 µg), doxycycline (30 µg), enrofloxacin (5 µg), sulfamethoxazole/trimethoprim (25 µg), amoxicillin (10 µg), and oxytetracycline (30 µg). *E. coli* ATCC 25922 was used as a quality control strain. The antibiotics tested were selected considering the antibiotics commonly used in the treatment of poultry with colibacillosis. APEC strains showing resistance to ≥3 classes of antibiotic were determined as multidrug resistant strains [21].

O-Serotyping

APEC strains with multidrug resistance (MDR) that were isolated from different production units were serotyped. For this, total of 30 APEC strains were used. To identify the serogroup of the APEC isolates, O-serogroup typing was performed by slide agglutination using all polyvalent and monovalent antisera provided by the manufacturer (Denka Seiken, Co., Ltd., Tokyo, Japan) [22].

Results

In this study, total of 99 APEC strains were isolated from chicken (21 broilers) and different types of husbandries (35 breeding and 43 hatchability). *E. coli* strains demonstrated high resistance to oxytetracycline and erythromycin, amoxicillin, doxycycline, sulfamethoxazole/trimethoprim and less resistance to enrofloxacin and florfenicol at rates of 82.83% (82/99), 75.76% (75/99), 67.68% (67/99), 59.69% (59/99), 38.38% (38/99) and 33.33% (33/99), respectively. Although the most common observed resistant phenotypes were determined against oxytetracycline, amoxicillin and erythromycin in all production units, the highest resistance determined 100% (5/5) and 92.3% (12/13) in broiler *(Table 1)*.

Many of the strains exhibited resistance to ≥3 of the seven antibiotics tested. According to the MDR profiles; 95.23% of strains isolated from broiler, 71.42% from breeding and 83.72% from hatchability. Multidrug resistance patterns of the strains to six antibiotic classes revealed that 18 APEC

Table 1. *Antibiotic resistance according to the production units*

Production Units	Antibiotics						
	Amphenicol	Tetracycline		B-Lactam	Sulfonamide	Macrolide	Fluoroquinolone
	Florfenicol % (n)	Oxytetracycline % (n)	Doxycycline % (n)	Amoxicillin % (n)	Sulfamethoxazole/ Trimethoprim % (n)	Erythromycin % (n)	Enrofloxacin % (n)
Broiler (n = 21)	85.71 (18)	100 (21)	85.71 (18)	95.23 (20)	90.47 (19)	95.23 (20)	90.47 (19)
Breeding (n = 35)	17.14 (6)	74.28 (26)	57.14 (20)	71.42 (25)	57.14 (20)	74.28 (26)	28.57 (10)
Hatchability (n = 43)	20.93 (9)	81.39 (35)	67.44 (29)	69.76 (30)	46.51 (20)	83.72 (36)	20.93 (9)
Total (n = 99)	33.33 (33)	82.83 (82)	67.68 (67)	75.76 (75)	59.69 (59)	82.83 (82)	38.38 (38)

strains were resistant to all of the antibiotics. We detected a rate of 18.18% and APEC strains showed multidrug resistance ≥3 antibiotic class rate of 81.81% *(Table 2)*.

Thirty multidrug resistance *E. coli* strains were selected and serotyped. The most common serogroups were O78 (43.3%) and O125 (16.6%) and the remaining 12 strains were O1 (6.6%), O18 (6.6%), O169 (6.6%), O8 (3.3%), O142 (3.3%), O143 (3.3%), O157 (3.3%), O158 (3.3%) and O164 (3.3%), serogroups *(Table 3, Table 4)*. O78 serogroup was found resistant to oxytetracycline, sulfamethoxazole/ trimethoprim, amoxicillin-doxycycline and erythromycin at the rates of 12/13 (92.3%), 11/13 (84.6%), 10/13 (76.9%), 9/13 (69.2%), respectively, and the O125 serogroup was resistant to erythromycin, oxytetracycline-doxycycline and sulfamethoxazole/trimethoprim, amoxicillin, at the rates of 5/5 (100%), 4/5 (80%), 3/3 (100%), respectively.

Discussion

Increasing antibiotic resistance is an important public health problem and resistance continues to spread due to many factors. Antibiotics are used for therapeutic and prophylactic purposes for broilers as stimulating growth in production. Some antibacterial drugs, such as ampicillin, tetracycline, chloramphenicol, enrofloxacin, neomycin, which are used in the treatment of *E. coli* infections to become resistant by inhibiting the microflora of the

Table 2. *Rate of APEC strains exhibiting multidrug resistance (MDR)*

Production Units	Resistance to ≥3 Antibiotics % (n)	Resistance to ≥4 Antibiotics % (n)	Resistance to ≥5 Antibiotics % (n)	Resistance to ≥6 Antibiotics % (n)	Resistance to ≥7 Antibiotics % (n)
Broiler (n=21)	95.23 (20)	95.23 (20)	95.23 (20)	90.47 (19)	66.66 (14)
Breeding (n=35)	71.42 (25)	60 (21)	48.57 (17)	14.28 (5)	8.57 (3)
Hatchability (n=43)	83.72 (36)	69.76 (30)	39.53 (17)	9.30 (4)	2.32 (1)
Total (n=99)	81.81 (81)	71.71 (71)	54.54 (54)	28.28 (28)	18.18 (18)

Table 3. *Relationship between O serogroups and production units*

Production Units	O Serogroups										
	O1 % (n)	O8 % (n)	O18 % (n)	O78 % (n)	O125 % (n)	O142 % (n)	O143 % (n)	O157 % (n)	O158 % (n)	O164 % (n)	O169 % (n)
Broiler (n =5)	-	-	-	40 (2)	20 (1)	-	-	-	20 (1)	-	20 (1)
Breeding (n =15)	6.6 (1)	-	13.3 (2)	60 (9)	13.3 (2)	-	6.6 (1)	-	-	-	-
Hatchability (n =10)	10 (1)	10 (1)	-	20 (2)	20 (2)	10 (1)	-	10 (1)	-	10 (1)	10 (1)
Total (n = 30)	6.6 (2)	3.3 (1)	6.6 (2)	43.3 (13)	16.6 (5)	3.3 (1)	3.3 (1)	3.3 (1)	3.3 (1)	3.3 (1)	6.6 (2)

Table 4. *Percentages and numbers of APEC strains in O78 (n=13) and O125 (n=5) displaying resistance to tested antibiotics*

O Serogroups	Antibiotics						
	Florfenicol % (n)	Oxytetracycline % (n)	Amoxicillin % (n)	Sulfamethoxazole/ Trimethoprim % (n)	Erythromycin % (n)	Enrofloxacin % (n)	Doxycycline % (n)
O78 (n = 13)	30.76 (4)	92.3 (12)	76.9 (10)	84.6 (11)	69.2 (9)	38.4 (5)	76.9 (10)
O125 (n = 5)	40 (2)	80 (4)	60 (3)	60 (3)	100 (5)	20 (1)	80 (4)

digestive system and these bacteria play an important role in the transfer of resistance genes and cause serious infections that affect the food chain [23,24]. For these reasons, since January 2006, legally importing feed additives containing antibiotics in Türkiye has not been allowed [25].

In this study, a high rate of antibiotic resistance was observed against antibiotics that are regularly used in treatment of poultry diseases in Türkiye. APEC strains regardless of breeding types of poultry demonstrated resistance to oxytetracycline and erythromycin at rate of 82.83%, amoxicillin with rate of 75.76%, doxycycline with rate of 67.68%, sulfamethoxazole/trimethoprim with rate of 59.69% and less resistance to enrofloxacin and florphenicol with rates of 38.38% and 33.33%, respectively. In our study, high tetracycline resistance is similar to previous studies [26] and supporting the findings of other studies conducted in other countries [3,11,13-16,27,28]. Jiang et al.[27] also reported that doxycycline is known as a type of tetracycline, resistance is higher than other antibiotics in China, but in our study tetracycline resistance follows the oxytetracycline. Gomis et al.[29] found as 61%, 65.2%, 65.2% resistance rates to erythromycin, tetracycline and doxycycline, respectively and reported that all isolates were susceptible to enrofloxacin. Al Agamy [30] reported that APEC strains were resistant to tetracycline and doxycycline at rates of 85% and 83%, respectively. Aggad et al.[31] found 45% enrofloxacin resistance and Li et al.[32] reported that resistance to florfenicol, sulfamethoxazole and trimethoprim, enrofloxacin 29%, 100% and 83%, respectively. Although different production units showed significant differences among antibiotic resistance profiles in our study, oxytetracycline resistance was still at the highest level. While the breeder and hatchery strains were significantly lower resistant against to enrofloxacin, sulfamethoxazole-trimethoprim and florfenicol; resistant strain rate was quite high in broiler. In particular, this situation showed that the antibiotics have not been used properly in broiler than other production types.

According to the results of multidrug resistance in APEC strains, 81.81% of APEC strains were found to be resistant to ≥3 antibiotics class, and 18.18% were resistant to all tested antibiotics. Several reports have concluded that MDR is more common than resistance to single antibiotic [33,34].

In the study, serotyping was performed on a total 30 MDR APEC strains and the majority of APEC serotypes obtained from all production units were identified as O78 as in other researches [11,13-16] and the second one is O125. Also, Dahshan et al.[35] reported that O125 serotype was among the most common serotypes in a study of stool and drinking water. Distribution of the remaining 12 strains were among O1, O8, O18, O142, O143, O157, O158, O164 and O169. This result is very important for better understanding the profile of APEC strains circulating nowadays in Türkiye, because no study was performed before on serotyping of APEC strains.

The highest antibiotic resistance in O78 and O125 serogroups were determined against oxytetracycline and erythromycin, respectively. The common APEC serotypes were determined as O78 (43.3%) and O125 (16.6%) and the others showed variability between 6.6% and 3.3%. Notable matters, according to these serotypes and resistance profiles were appeared in same integration, also hatchability. These findings are consistent with other reports where diseases caused by APEC strains in both parent flocks and their progeny and spread from breeders to hatcheries on the same farm via environmental contamination or vertical transmission [36-39]. On the other hand, Da Silveira et al.[40] and Da Silveira et al.[41] reported that opportunistic *E. coli* strains could be pathogenic for one day old chicks crossing the egg barriers during the egg laying process and also they could be potential pathogens via plasmids (transfer of virulence factors) after hatching.

As a result, biochemical and serological characterization and antibiotic sensitivity of APEC strains were determined in the integrated poultry production diagnosed with avian colibacillosis. The results obtained from this study suggest that transmission of APEC agents and also antibiotic resistance genes belong to these agents also from broods to broiler chickens could be possible. Thus, APEC strains should be subjected to the antimicrobial susceptibility test for appropriate antibiotic selection in the treatment of the disease.

Availability of Data and Materials

Data supporting the study findings are available from the corresponding author on reasonable request (N. Karacan Sever).

Ethical Approval

The data for this study were collected from 'dead animals or their tissue', so the study does not require any ethical approval.

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

NKS, OSY and MA planned and designed the study. NKS and OSY performed the experiments; NKS, OSY and MA contributed to the analysis and interpretation of data. NKS drafted the manuscript. All authors read and approved the final manuscript.

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