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Short Communication

Antibioresistance of *Escherichia coli* Strains Isolated from Chicken Colibacillosis in Western Algeria

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Abstract: In western Algeria, 251 *Escherichia coli* isolates were recovered from broilers with clinical signs and lesions of colibacillosis. Serotyping showed that 82% of the isolates belong to one of the following serotypes: O78, O2, and O1. Antibiograms revealed a high level of resistance to oxytetracycline (82%), amoxicilline and ampicilline (47%), trimethoprim-sulfmethoxazole (42%), oxolinic acid and flumequine (31%). However, only a low percentage of strains were resistant to enrofloxacine (6%) and colistine (3%). Although multiresistance was common, low association was found between the serotype and antibioresistance.

Key Words: *Escherichia coli*, antibioresistance, colibacillosis, Algeria, poultry

Among bacterial infections, colibacillosis is a worldwide major cause of morbidity and mortality in poultry and responsible for significant economic losses to the poultry industry. Stordeur and Mainil (1) reported that the causative bacteria, avian pathogenic *Escherichia coli* (APEC), induce various syndromes including respiratory tract infection (airsacculitis), acute colisepticemia, salpingitis, and cellulitis. The most common form of colibacillosis occurs in 3- to 10-week-old chickens. It is characterized by an initial respiratory infection usually induced by mycoplasmal and/or viral infections and enhanced by a high level of ammoniac in poultry houses. The disease evolves as a systemic infection (perihepatitis, pericarditis, and septicaemia) due to the invasive abilities of the *Escherichia coli* strains (2). Numerous studies have shown that APEC strains usually belong to serogroups O1, O2, and O78 (3,4) but other serogroups can also be identified.

In Algeria, avian colibacillosis is responsible for large economic losses in poultry breeders resulting in low performances, weight loss, delayed onset of egg production, and mortality. This has led to an intensive use of antibiotics resulting in an unavoidable loss of the efficacy of treatments. In the absence of epidemiologic data allowing a survey of microbial resistance to various antibiotics, the choice of antibiotic for treatment remains quite arbitrary. Indubitably, there will be an effort to get a better knowledge of the level of antibioresistance status of APEC in western Algeria. Thus, we have collected and characterized strains from cases of avian colibacillosis.

The study was conducted in western Algeria (Oran, Mostaganem, Tiaret). Thirty large capacity broiler chick flocks, between 20,000 and 30,000 broilers, were selected. Eight chicken were taken by simple randomization during each of 35 colibacillosis outbreaks from clinically affected broilers (weight loss, acute septicaemia, osteomyelitis, and sudden death) and showing characteristic lesions at necropsy (enteritis, pericarditis, airsacculitis, perihepatitis, and synovitis). This represents 8 to 24 samples per farm.

In each sampled case one tissue liver, spleen, or heart blood was taken (1) and cultured on Drigalski agar (Sanofi-Diagnostics Pasteur, France) and incubated aerobically at 37 °C for 18 to 24 h. Suspected *E. coli* colonies were subsequently inoculated on eosine-methylene blue agar (Bio Mérieux, France) and incubated aerobically at 37 °C for 18 to 24 h. The isolates that are gram-negative, catalase positive, oxidase negative, and have a dark green, black metallic sheen on eosin-methylene blue agar (5) were identified as *E. coli* using

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the API system (Bio Mérieux, France) for the identification of Enterobacteriaceae. A total of 251 *E. coli* isolates were collected from 4- to 7-week-old broilers.

Serotype was determined by agglutination test using specific antiserum raised against O1:K1, O2:K1, and O78 antigens (Biovac, Angers, France andLDA22, Ploufragan, France) according to Finazzi et al. (6).

Antibiotic sensitivity was determined by the disc diffusion method on solid Mueller-Hinton medium (Sanofi-Diagnostics Pasteur, France) according to the guidelines of the "Comité de l'Antibiogramme de la Société Française de Microbiologie". Standard paper disks containing antibiotics widely used in chickens in Algeria; oxytetracycline (30 µg), ampicillin (10 µg), amoxicilline (20 µg), trimethoprim-sulfamethoxazole (25 µg), oxolinic acid (10 µg), flumequine (30 µg), enrofloxacine (5 µg), colistine (10 µg) were laid on the medium. Commercial antibiotic disks were purchased from Sanofi-Diagnostics Pasteur (Marnes-la-Coquette, France) except enrofloxacin which was provided by Bayer (Leverkusen, Germany). The plates were incubated for 24 h at 37 °C and inhibition zones were measured.

Pearson coefficient correlation was used to compare the frequency of associated antibioresistance.

The observed lesions at necropsy were characteristic of colibacillosis and were in decreasing frequency: pericarditis, perihepatitis, tracheitis, airsacculitis, and nephritis.

E. coli O78, O2:K1, and O1:K1 represented 110 (44%), 73 (29%), and 23 (9%) of the isolates, respectively. The remaining 45 (18%) of the isolates did not belong to these 3 serotypes.

The resistance frequencies (RFs) for each antibiotic tested are shown in Table 1. These RFs are high and permit the division of the antibiotic into 3 groups.

The first group (I) included the antibiotics to which there was a high level of resistance: oxytetracycline, ampicillin, amoxicilline, and trimethoprim-sulfamethoxazole. The second group (II) includes the antibiotics to which there was a medium level of resistance, oxolinicacid and flumequine. The third group (III) contains the antibiotics to which there was a low level of resistance. Significant differences in the resistances associated with the serotypes were only observed for oxytetracycline and ampicillin. Strains belonging to the O78 serotype or to nonidentified serotypes were more frequently resistant to oxytetracycline and/or to ampicillin compared to O1:K1 and O2:K1 strains.

The percentage of resistant isolates was high (98%). Ninety three percent of the isolates were resistant to at least 2 antibiotics and 22% were resistant to at least 4 antibiotics. About 10 % of isolates were resistant to 5 or 6 antibiotics (Table 2). The correlation between multiresistance and serotypes, as calculated with Pearson coefficient, was not significant; a higher positive correlation (r = 0.44) was found for oxytetracyclin and ampicillin.

Table 1. Antibioresistance of *E. coli* strains isolated.

	Number of resistant strains (%)				
Antibiotic	O78	O2:K1	O1:K1	Other serotypes	Total
Oxytetracycline	104 (95)	50 (69)	13 (57)	39 (87)	206 (82)
Ampicillin	63 (57)	29 (40)	5 (22)	21 (47)	118 (47)
Amoxicilline	63 (57)	28 (38)	6 (26)	21 (47)	118 (47)
Trimethoprim-sulfmethoxazole	54 (49)	23 (31)	7 (30)	22 (49)	106 (42)
Oxolinic acid	36 (33)	23 (31)	5 (22)	15 (33)	79 (31)
Flumequine	36 (33)	23 (31)	5 (22)	14 (31)	78 (31)
Enrofloxacine	7 (6)	4 (5)	2 (9)	1 (2)	14 (6)
Colistine	2 (2)	2 (3)	2 (9)	1 (2)	7 (-3)
Total number of isolates	110	73	23	45	251(100)

Table 2. Strains of *E. coli* showing multiresistance.

Number of antibiotics	Percentage of strains resistant out of 8 tested
0	2
1	6
2	25
3	45
4	12
≥5	10

A total of 52 antibiotypes could be distinguished. The most frequent are those designated in Table 3 as i, h, b, k, and c.

A high level of multiresistance (22.7%) was observed for 3 antibiotics: 9.16 for ampicillin-oxytetracycline-amoxicilline and 13.54 for ampicillin-flumequine-oxytetracycline. In group I, a high resistance to 3 antibiotics largely used in the studied farms has been retained: oxytetracycline, ampicillin, and amoxicilline (Table 1). In view of the whole range of antibiotics available in Algeria and the lack of legislative restrictions on their use for therapy, prophylaxis, or growth promotion, the globally high incidence of antibiotic resistance observed in the present study is not surprising. Resistances to ampicillin, trimethoprim-sulfamethoxazole, oxolinic acid, and flumequine were far higher compared to previous studies (3,4,7). The percentage of resistant bacteria to oxytetracycline was the highest (82%), as observed in Marroco by Filali et al. (8), and thus used by breeders very frequently. However, resistance to enrofloxacine and colistine remained low, reflecting the infrequent use of this antibiotic in poultry breeding in Algeria. Multiresistance appeared as a veritable problem as the majority of strains (63.7%) was resistant to at least 2 antibiotics. Indeed, numerous antibiotics are administrated often concomitantly for prophylaxis or in infections. This indicates that the abusive and indiscriminant use of antibiotic is probably at the origin of the high incidence of antibioresistances and multiresistances of *E. coli* in poultry breeding in western Algeria. Such practices, especially without prior antibiotic sensitivity testing of bacterial isolates, may lead to the development of a pool of antibiotic-resistant genes and to the selection of increasing numbers of resistant *E. coli* clones. This first study should constitute a basic reference for further surveys of antibiotic-resistance of *E. coli* isolates in western Algeria. The evolution of antibiotic-resistance of avian *E. coli*, together with the evolution of therapeutic practices, should be controlled by a network of epidemiological survey of poultry breeding in Algeria.

Table 3. The most frequent antibiotic resistance patterns in *E. coli* strains.

Resistance patterns	Designation	Percentage of strains
OXA	A	3.58
OT.TMS	B	6.77
AMP.OT	C	4.38
OXA. OT	D	2.39
AMO.OXA	E	2.39
AMP.TMS. OT	F	3.98
OT.AMO.TMS	G	2.39
AMP. OT.AMO	H	9.16
AMP.FLU. OT	I	13.54
AMP.TMS.OXA	J	2.78
TMS. OT.AMO	K	4.78
OT.AMP.AMO.TMS	L	2.39
AMO.OT.OXA.FLU	M	2.39
AMO.TMS. OT.ENR	N	2.39
FLU.AMP.AMO.OT.OXA	O	3.98
TOTAL		67.33

OXA oxolinic acid, OT oxytetracycline, TMS trimetoprim-sulfamethoxazole, AMP ampicillin, AMO amoxicilline, FLU flumequine, and ENR enrofloxacine.

In conclusion, this work demonstrates alarming high individual and multiple resistances to antibiotics in *E. coli*. The problem of antibiotic-resistances of avian *E. coli* isolates is of particular importance in Algeria where exists a high risk of human contamination because of manual slaughtering of animals. Antibiotic resistances are frequently encoded by conjugative plasmids or transposons, thus *E. coli* of avian origin could act as a possible source for the transfer of antibiotic resistances to other bacterial species including human pathogens (9,10). Thus, an increase in the reservoir of antibiotic resistant bacteria could heavily impair the treatment of human diseases.

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