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Antimicrobial Resistance of *Escherichia coli* Isolated from Chickens with Colibacillosis

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Abstract: In order to asses susceptibility of *Escherichia coli* to seven antimicrobial drugs, we tested one hundred *Escherichia coli* isolates, recovered from broilers with clinical signs and lesions of colibacillosis in the West area of Algeria. Serogrouping showed that 52% of the isolates belong to one of the serotypes O78, O2 and O1. Antibiograms revealed a high level of resistance to oxytetracycline, trimethoprim-sulfmethoxazole and enrofloxacin (87, 70 and 45%, respectively). A low percentage of strains were resistant to gentamycin and nitrofurane (3 and 2%, respectively). All strains were susceptible to ampicillin. 72% of the isolates were resistant to at least 2 antibiotic and 45% were resistant to at least 3 antibiotics.

Key words: *Escherichia coli* · Resistance · Antibiogram · Algeria · Lesions · Serotype · Poultry

INTRODUCTION

Avian colibacillosis is responsible for large economic losses in poultry rearing resulting in low performances, weight loss, onset of egg production and mortality. Avian pathogenic (APEC) *Escherichia coli* causative bacteria of colibacillosis, induces various syndromes including respiratory tract infection (airsacculitis), acute colisepticemia, salpingitis and cellulitis [1]. The most common form of colibacillosis occurs among 2 to 10 week-old chickens.

Antimicrobial therapy is an important tool in reducing both the incidence and mortality associated with avian colibacillosis [2]. This has increased resistance to commonly used antimicrobials both in the public health and veterinary sectors.

Antimicrobial-resistant *Escherichia coli* and others pathogenic bacteria can be transferred from animals to humans through consumption of contaminated food and food products and thus present a public health risk.

Several studies have shown that APEC strains usually belong to serogroups O1, O2 and O78 [3, 4] but other serogroups have also be identified.

In Algeria, bacterial infections continue to be treated without first establishing an antibiogramm.

In the absence of epidemiologic data allowing a survey of antibiotic resistances, the use of antibiotics remains quite irrational. The present work was conducted to estimate the antimicrobial resistance of *Escherichia coli* isolates from chicken in western Algeria.

MATERIALS AND METHODS

Sampling Site and Procedure: The study was conducted in Western Algeria. Eighty four one to eight week-old broiler with possible colibacillosis collected in different farms from Tiaret and Tlemcen Departments were autopsied.

Autopsy: Organs showing characteristic lesions related with colibacillosis were inspected and random samples were taken for further analysis.

Culture and Biochemical Characterisation: Visceral organs liver and spleen were cultured on Brain Heart Infusion agar (Biochemika, Spain) and incubated aerobically at 37°C for 18 to 24h. Suspected *Escherichia coli* colonies were subsequently inoculated on Hecktoen agar (Pasteur Institute, Algeria) and incubated as above. The identification of *Escherichia coli* was based on the results of diagnostic tests, including Gram stain, catalase and oxidase [5].

Metabolic profiles were analysed for each isolate using API system (Bio Mérieux, France) used for the identification of *Enterobacteriaceae.*

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Serogrouping. Serogroup was determined by agglutination test with specific antiserum raised against O1, O2 and O78 antigens (Biovac, Angers, France) according to Finazzy *et al.* [6].

Antimicrobial Sensitivity: Antibiotic sensitivity was determined by the disc diffusion method on solid medium of Mueller-Hinton (Biochemika, Spain) according to the guidelines of the National Committee for Clinical Laboratory Standards [7].

Ampicillin, trimethoprim-sulfamethoxazole, enrofloxacine, colistine, nitrofuranes, tetracycline and gentamycine standard paper disks were laid on the medium.

Commercial antibiotic disks were purchased from Bioanalyse, France (gentamycine, enrofloxacine, trimethoprim-sulfamethoxazole); Bio-Rad, France (ampicilline, tetracycline); Himedia, Inde (nitrofuranes). Colistine was provided by Oxoid, England. The plates were incubated for 24 h at 37°C and inhibition zones measured. *Escherichia coli* ATCC 25922 was used as reference strain.

RESULTS

Post-Mortem Examination: The observed lesions at necropsy were characteristic of colibacillosis and were in decreasing frequency: spleen congestion (66%), perihepatitis (62%), airsacculitis (40%), enteritis (32%) and pericarditis (31%). Culture, biochemical and serological identification. Isolates were catalase positive, oxydase negative) and colony were round, salmon with smooth aspect on Hecktoen agar. The API commercial differentiation system identified 100 isolated strains as *Escherichia coli.*

Only 48 % of the isolated strains belonged to the three serogroups with 21 to O78, 16 to O2 and 15 to O1 (Table 1).

Resistance Frequencies: The resistance frequencies (RF) for each antibiotic tested are shown in Table 2. Most of the strains were resistant to oxytetracycline and to trimethoprim-sulfamethoxazole with 87 and 70%, respectively. However, resistance to enrofloxacin was elevated (45%). Resistance to gentamycine and nitrofurane was infrequent (3 and 2%, respectively). All isolated strains were susceptible to ampicilline.

All strains were more frequently resistant to oxytetracycline, trimethoprim-sulfamethoxazole and enrofloxacin.

Multiresistance: The percentage of multiresistant isolates was high: 89%. A total of 72% were resistant to at least 2 antibiotic and 45% were resistant to at least 3 antibiotics. Less than 10% of isolates were resistant to four or five antibiotics (Table 2). However, the trend of resistance of isolates for oxytetracyclin and trimethoprim-sulfamethoxazole were more elevated followed by enrofloxacine and colistine than gentamycine and nitrofuranes.

Table 1: Antibioresistance of isolated *Escherichia coli* strains

	Number of resistant strains (%)				
Antibiotic (µg)	O78	O2	O1	Other serotypes	Total
Oxytetracycline (30)	20 (95)	16 (100)	9 (60)	42 (87)	87
Trimethoprim-sulfamethoxazole (25)	19 (90)	10 (62)	9 (60)	32 (67)	70
Enrofloxacine (50)	18 (85	5(31)	5 (33	17 (35)	45
Colistine (10)	1 (5)	0	4 (27)	8 (17)	13
Gentamycine (10)	0	0	1 (7)	2 (4)	3
Nitrofuranes (30)	0	1 (6)	1 (7)	0	2
Ampicillin (10)	0	0	0	0	0
Total number of isolates	21	16	15	48	100 (100)

Table 2: Strains of *Escherichia coli* showing multiresistance

Number of antibiotics out of 7 tested	Percentage of strains resistant
0	11
1	17
2	27
3	36
4	5
5	4

Table 3: Most frequent antibiotic resistance patterns in *Escherichia coli* strains

Resistance patterns	Designation	Percentage of strains
OT	A	15
OT.TMS	B	23
OT.CT	C	3
OT.TMS. ENR	D	35
OT.TMS.CT. ENR	E	4
OT.TMS.CT.G. ENR	F	3

OT oxytetracycline, TMS trimetoprim-sulfamethoxazole, CT colistine
ENR enrofloxacine, G gentamycine, N nitrofuranes

Antibiotypes: A total of 12 antibiotypes could be distinguished. The most frequent are those designated in Table 3 as D, B and A. A total (15 %) of strains were resistant only for oxytetracycline. A percentage of 23% of strains were resistant to oxytetracycline. trimetoprim-sulfamethoxazol. More than the third of the isolates (35%) were resistant for 3 antibiotics: oxytetracycline. trimetoprim-sulfamethoxazol. enrofloxacine. 4 and 3% of the strains present resistance to 4 (oxytetracycline.trimetoprim-sulfamethoxazol. colistine. enrofloxacine) and 5 (oxytetracycline. trimetoprim-sulfamethoxazol. colistine.gentamycine. enrofloxac- ine) antibiotics, respectively (Table 3).

DISCUSSION

A high resistance has revealed to three antibiotics: oxytetracycline, trimethoprim-sulfamethoxazole and enrofloxacin (Table 1). Considering the numerous types of antibiotics available in Algeria and the lack of organization and restrictions on their use, the higher incidence of antibiotic resistance observed in this present study is predicable and is consistent with results obtained in Dakar [8] whereas all strains were resistant to one or more antibiotics.

The percentage of resistant bacteria to oxytetracycline was the highest (87%) as observed in Morocco [9]. Resistance to trimethoprim-sulfamethoxazole is similar to this reported by Blanco, *et al*. [3] was far higher than in other studies [3,4,10]. However, resistance to gentamycine (similar to the reported in Iran [11]) and nitrofuranes remained at a low level, reflecting the unfrequent use of these antibiotics in poultry rearing in Algeria.

Multiresistance appeared as a veritable problem as the majority of strains (72%) was resistant to at least two antibiotics.

Compared with a previous study [12], our results showed that the previous dominant serotypes (O78, O2 and O1) represented less than half the isolates in this study. Resistance to oxytetracycline, trimethoprim-sulfmethoxazole and enrofloxacin is increasing. Resistance to ampicillin disappeared; this antibiotic is currently not used considering its high cost.

Blind antimicrobial therapy, excessive usage of antimicrobial agents for prophylaxis and inappropriate treatment may explain the high incidence of antibioresistances and of multiresistances of *Escherichia coli* in poultry rearing 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 *Escherichia coli* clones. *Escherichia coli* of avian origin could act as a possible source for the transfer of antibiotic resistances to other bacterial species including human pathogens [13].

In conclusion, antimicrobial resistance is an important factor challenging the poultry industry in Algeria whereas it exists a high risk of human contamination because of manual slaughtering of animals. Thus, an increase in the reservoir of antibiotic resistant bacteria could heavily impair the treatment of human diseases. Further studies are necessary in order to determine bacterium mechanisms of resistance. There is a need to a national veterinary surveillance network monitoring resistance to antimicrobial agents in the main pathogenic bacteria isolated from diseased poultry, cattle and sheep.

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