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Antimicrobial susceptibility of *Escherichia coli* strains isolated from broiler chickens affected by colibacillosis in Setif

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In Setif East Algeria, one hundred and eighty *Escherichia coli* strains were isolated from broiler chickens with colibacillosis and were examined for susceptibility to antimicrobials of veterinary significance. *In vitro* antibiotic activities of 12 antibiotic substances against the isolates were determined by disc diffusion test. Antibiogram revealed a high level of resistance to enrofloxacin (72.2%), neomycin (75%), trimethoprim-sulfmethoxazole (82.2%), ampicillin (84.5%) and amoxicillin (87.8%), nalidixic acid (96.7%) and doxycycline (98.3%). There were moderate levels of resistance to chloramphenicol (45.6%) and streptomycin (66.1%). There were low levels of resistance to nitrofurantoin (18.9%), colistin (5.5) and gentamicin (5.5%). All strains were multi-drug resistant and more than half (56.1%) of the isolates were resistant to eight antibiotics. Thus, 60 antibiotic resistant patterns in *E. coli* strains were isolated, of which 13 were present significantly. These findings suggest the need for the introduction of surveillance programs in Algeria to monitor antimicrobial resistance in pathogenic bacteria that could be potentially transmitted to humans from animal food.

Key words: *Escherichia coli*, antibioresistance, colibacillosis, Algeria, poultry.

INTRODUCTION

Avian colibacillosis is a bacterial disease caused by avian pathogenic *Escherichia coli* (APEC) strains, (Stordeur and Mainil., 2002; Robineau and Moalic., 2010). Colisepticemia and air sacs disease are the most common forms of colibacillosis in broiler chickens (Stordeur and Mainil., 2002; Saif, 2003). Colibacillosis is an economical devastating disease in the poultry industry; it is the primary cause of morbidity, mortality and condemnation of carcasses in Algeria and many parts of the world (Dho-Moulin and Fairbrother, 1999; Hammoudi and Aggad., 2008; Ewers et al., 2003). It is likely the primary cause of antibiotic treatment in farms, and the emergence of resistant strains is a legitimate concern (Robineau and Moalic, 2010). Resistance to two or more classes of antibiotics is now common place in veterinary medicine, and the choice of antibiotic for treatment remains quite arbitrary. Very little data are available in epidemiology of antimicrobial resistance of *E. coli.* Hence the aim of this study is to get a better knowledge of the level of antibioresistance and to provide a baseline of antimicrobial resistance among these pathogens for future studies. The present study determines antimicrobial susceptibility among a collection of avian pathogenic *E. coli,* recovered from diseased birds diagnosed with

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

Antibiotic	Number of resistant strain (%) (n=180)
Amoxicillin (AMX,25 µg)	158 (87.8)
Ampicillin (AM, 10 µg)	152 (84.5)
Doxycycline (DO, 30 µg)	177 (98.3)
Nalidixic acid (NA, 30 µg)	174 (96.7)
Enrofloxacin (ENR, 5 µg)	130 (72.2)
Gentamycin (G, 10 µg)	10 (5.5)
Streptomycin (S, 30 µg)	119 (66.1)
Neomycin (N,30 µg)	135 (75)
Colistin (CL, 50 µg)	10 (5.5)
Nitrofurantoin (FT, 300 µg)	34 (18.9)
Chloramphenicol (C, 30 µg)	82 (45.6)
Trimethoprim-sulfmethoxazole (CO, 25 µg)	148 (82.2)

colibacillosis in East Algeria (Setif).

MATERIALS AND METHODS

Sample collection

From 30 May to 15 August, 2010, 200 chicken carcasses were collected from the slaughter chain. The animals came from the Regional Office Poultry of the East; they were at the end of production, that is, between 49 and 56 day old. The strain was ISA broiler F15. Autopsies were performed in the slaughter house. 7800 broilers were provided for slaughter. Samples were collected weekly each Sunday. From 300 broilers with growth retardation or generalized carcass congestion, 20 were randomly selected and presented at autopsy, expressing lesions of airsacculitis, pericarditis, perihepatitis and congestion of organs that resemble the colibacillosis form. All samples (200 livers) were taken using sterilized utensils and placed in separate sterile plastic bags to prevent spilling and cross contamination. Then they were immediately transported to the laboratory in a cooler with ice packs (+4°C).

Bacteriological analysis

The samples were processed immediately upon arrival using aseptic techniques. Briefly, isolation and identification of *E. coli* was done according to the protocol recommended by Livrelli et al. (2007). Shortly, the organs were flamed using a Bunsen burner and cut into small dice. Enrichment was done by seeding cubes in tubes of BHIB (Pasteur Institute of Algeria) and incubated aerobically at 37°C for 18 to 24 h. A drop of broth was inoculated with BHIB method of exhaustion on Mc Conkey medium (Bio Lab, Algeria) (selective medium for Gram-negative enterobacteriaceae) and then incubated aerobically at 37°C for 18 to 24 h. Positive colonies have a diameter of 2-3 mm, light pink color (lactose +) and are surrounded by an opaque halo due to precipitation of bile salts. They are Gram negative, catalase positive and oxidase negative. These colonies were identified as *E. coli* using the API system (Bio Mérieux, France) for the identification of Enterobacteriaceae.

Antimicrobial susceptibility

Antibiotic sensitivity was determined by disc diffusion method (Bauer et al., 1966) on solid Mueller-Hinton medium (Pasteur institute of Algeria) according to the guidelines of the "National Committee for Clinical Laboratory Standards". *E. coli* ATCC 25922 was used as control. Susceptibility was tested against the following antibiotics discs: Ampicillin (AM 10 µg), amoxicillin (AMX, 25 µg), chloramphenicol (C, 30 µg), colistin (CL, 50 µg), Streptomycin (S, 10 µg), Neomycin (N, 30 µg), Nalidixic acid (NA, 30 µg), Enrofloxacin (ENR, 5 µg) , doxycycline (DO, 30 µg), nitrofurantoin (FT, µg), trimethoprim/sulfamethoxazole (CO, 25 µg) and gentamycin (G, 10 µg). Commercial antibiotic disks were purchased from Bio Mérieux, France (ampicillin, amoxicillin, chloramphenicol, colistin, streptomycin, and neomycin); Himedia, India (gentamycin, trimethoprim/sulfamethoxazole, nitrofurantoin); Oxoid, England (doxycycline, nalidixic acid, enrofloxacin). The plates were incubated for 24 h at 37°C and the diameters of inhibition zones were interpreted by referring to the reading table of Enterobacteria as recommended by the Antibiogram Committee of the French Society for Microbiology (CA-SFM, 2010).

RESULTS AND DISCUSSION

One hundred and eighty *E. coli* strains were collected from 200 samples of livers; the susceptibility of these strains to each antimicrobial agent tested is shown in Table 1. All isolated *E. coli* showed multi-drug resistance, and multi-drug resistance was observed in all of the isolates as shown in Table 2. A total of 60 anti-biotypes were distinguished. The most frequent are those designnated in Table 3 as H, G and D. A high level of multiresistance (24.4%) was observed against 9 antibiotics. The most common multi-drug resistant profile among these isolates was profile H (19.4%), which was resistant to ampicillin, amoxicillin, doxycycline, nalidixic acid, Trimethoprim/Sulfamethoxazole, neomicyne, chloramphenicol, Enrofloxacine, and streptomycine. Co-resistance to ampicillin, amoxicillin, doxycycline, nalidixic acid, trimethoprim/sulfamethoxazole and neomycin was found in eleven of the thirteen most common multi-drug resistant patterns.

Among the 200 chicken carcasses autopsied, expressing lesions of colibacillosis, bacteriological analysis of livers got 180 positive cultures of *E. coli* (90%). In the remaining 20 cases (10%), bacterial cultures were negative. The negative cultures may result from drug interven-

Table 2. Strains of *E. coli* showing multi-drug resistance.

Number of antibiotic	Number of resistant starin	Percentages (%) resistant strain
0	0	0
1	0	0
2	2	1.1
3	9	5.0
4	12	6.7
5	7	3.9
6	14	7.8
7	35	19.4
8	42	23.3
9	44	24.4
10	10	5.6
11	3	1.7
12	2	1.1

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

Resistance pattern	Designation	Number of strain (%)
Do-Am-Amx	A	4 (2.2)
Am-Enr-Co-Amx-Na-Do	B	8 (4.4)
Am-Amx-Do-Na-Co-N-S	D	18 (10)
Am-Amx-Do-Na-Co-N-Enr	C	5 (2.8)
Am-Amx-Do-Na-Co-N-Enr-S	E	13 (7.2)
Am-Amx-Do-Na-Co-N-S-Ft	F	3 (1.7)
Am-Amx-Do-Na-Co-N-Enr-C	G	18 (10)
Am-Amx-Do-Na-Co-N-C-Enr-S	H	35 (19.4)
Am-Amx-Do-Na-Co-N-Ft-S-C-Enr	I	10 (5.6)
Am-Amx-Do-Na-Co-N-Enr-S-C-Ft-Cl-	J	1 (0.6)
Am-Amx-Do-Na-Co-N-Na- Enr-S-G-Ft-Cl	K	1 (0.6)
Am-Amx-Do-Na-Co-N-S-G-C-Ft-Cl	L	1 (0.6)
Am-Amx-Do-Na-Co-N-Enr-S-G-C-Ft-Cl	M	2 (1.1)

tion before referring the cases to the laboratory as reported by Saberfar et al. (2008). The antibiotics, which were highly resisted, are in ascending order: Streptomycin (66.1%), enrofloxacin (72.2%), neomycin (75%), trimethoprim/ sulfamethoxazole (82.2%), ampicillin (84.5), amoxicillin (87. 8%), nalidixic acid (96.7%) and doxycycline (98.3%). The abusive and anarchic use of the whole range of antibiotics available in Algeria is probably the major cause of the high percentages of resistance. In addition, lack of legislative restrictions of their use for therapy, prophylaxis, or growth promotion can be seen in the study of Hammoudi and Aggad (2008).The antibiotics, that were moderately resisted, are nitrofurans (18.9%) and chloramphenicol (45.6%); despite the fact that administration of chloramphenicol and nitrifurantoin is forbidden in veterinary, resistance to these antibiotics was high. This is probably due to persistence of previous resistances or more likely to illegal use of these agents. The antibiotics that had low levels of resistance from the strains are gentamycin and colistin (5.5%). The low level of resistance to colistin could be explained by the moderate use of this molecule in poultry. This molecule does not cross the intestinal barrier and is inactive orally on systemic colibacillosis. On the other hand, the resistance of Gram-negative bacteria is uncommon to colistin, even exceptional, and is chromosome-type. However, Garnacho-Montero et al. (2003) reported that chromosomal mutation is rare. The high sensitivity of the *E. coli* strains to gentamycin is because this antibiotic is not used. We explain 5.5% rate of natural resistance of *E. coli* to gentamycin. It is not available for poultry in Algeria and is used only for humans. Resistance was observed in all of the examined strains. This is similar to the findings of previous studies done in other countries by Blanco et al. (1997), Zahraei and Salehi (2006) and Saberfar et al. (2008). But our results were higher than those reported by Hammoudi and Aggad (2008) and Aggad et al. (2010) in Western Algeria.

Multi-resistance appears as a veritable problem, as 100% of *E. coli* strains were resistant to two antibiotics at least, while over three quarters (75.5%) were resistant to 7 antibiotics at least; more than half (56.1%) were resistant to at least 8 antibiotics and less than a third (32.8%) were resistant to at least 9 antibiotics. Indeed, numerous antibiotics are administrated often concomitantly for prophylaxis or infections. This indicates that the abusive and indiscriminate use of antibiotic is probably the genesis of the high incidence of antibioresistances and multiresistances of *E. coli* in poultry breeding in Eastern 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, as reported by Hamoudi and Aggad (2008). Lafont et al. (1984) and Chulasiri and Suthienkul (1989) reported that characteristics of virulent *E. coli* in birds and other animals are often shared, and avian strains potentially can be a source of genes and plasmids that encode for antimicrobial resistance and virulence factors. In our study, co-resistance to ampicillin, amoxicillin, doxycycline, nalidixic acid, trimethoprim/sulfamethoxazole-neomycin was particularly highlighted. It is present in 11 out of 13 most important multidrug resistant profiles. More than half of *E. coli strains* (56.8%) express this co-resistance. According to the study of Courvalin (2008), the consequence of this genetic organization is co-selection: a class of antibiotics to which the bacteria are resistant may select resistance to unrelated classes of antibiotics, thus generating a wide resistant phenotype of bacterium. The results of this multiresistance were treatment failures, and consequently reduced production due to high levels of morbidity and mortality in poultry flocks. We must consider the danger of *E. coli* strains expressing antibiotic resistant patterns I, J, K, L and M, with rates of 5.6, 0.6, 0.6, 0.6 and 1.1% respectively. These strains show resistance to 10, 11 and 12 antibiotics, even to conventional antibiotics regarded as the most active on *E. coli* strains, such as gentamicin, colistin and nitrofurans. These strains could transfer their wide antibiotic resistant pattern via the exchange of genetic material. Bebora et al. (1994) and Davies (1994) indicated 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. Thus, an increase in the reservoir of antibiotic resistant bacteria could heavily impair the treatment of human diseases. Van Den Boogard et al. (2001) identified similar antibiotic resistant patterns present in *E. coli* isolated from people who worked with these birds, and, in some instances, specific strains were shared among the birds and workers. These findings indicate that transmission of resistant organisms or plasmids from poultry to people is common.

Conclusion

The indiscriminate use of antibiotics by farmers without veterinary advice is a practice that is becoming increasingly common. This practice determines the selection of resistant bacteria and the increase in multidrug resistance. In our study, alarming rates were observed for individual and multiple antimicrobial resistant strains of *E. coli* against the majority of antibiotic molecules commercially available legally, making them ineffective in the fight against *E. coli*. This causes economic losses in farms, related to mortality and high morbidity and seizures in slaughterhouses. The problem of antibiotic-resistances of avian *E. coli* isolates is of particular importance in Algeria. A high risk of human contamination exists due to the manual slaughtering of animals.

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