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Antimicrobial Resistance of *Escherichia coli* Isolated from Chickens in West of Algeria

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Abstract:

Modern poultry flocks undergo strong microbial pressure. Antibiotics can contribute to reduce bacterial infections. Their use increased these last years. Studies performed in Morocco and Algeria highlighted the importance of antibioresistance after excessive use of antibiotics in poultry breeding. In western Algeria, 240 strains of enterobacteriaceae were isolated according to usual bacteriological procedures. In order to assess antimicrobial resistance, the disc diffusion method for antibiotic susceptibility (tetracycline (TE), enrofloxacin (ENR), trimethoprim+sulfamethoxazole (SXT), amoxicillin+clavulanic acid (AMC), ceftiofur (KF), colistin (CT), neomycin (N), gentamicin (GN) and chloramphenicol (C) was applied (Antibioresistance Committee of the French Microbiology Society, 2010). All enterobacteriaceae strains isolated presented at least one resistance to those antibiotics. *Escherichia coli* counted for 47.5% of these strains (N=114). By omitting intermediate resistances, 28% of *E. coli* presented a resistance to at least 6 antibiotics and 31.6% to 5 antibiotics. In general, 90.35%, 79.82%, 70.17%, 92.10%, 62.28%, 31.57% and 21.05% of *E. coli* were resistant to, respectively, TE, ENR, SXT, AMC, KF, CT and N. Considering such a high resistance rate, it is strongly advised to implement epidemiological survey of bacterial resistances at the regional level.

Keywords: *Escherichia coli*, Poultry, Antibiotic, resistance, West Algeria

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1. Introduction

Antibiotic resistance is a growing problem in veterinary public health. The threat to public health from the transfer of pathogens comes from animals to humans through indirect contact such as food or by direct contact with the animal. In addition, another potential danger is the transfer of resistance from animals to humans by commensal bacteria such as *Escherichia coli*.

Among the bacterial infections, colibacillosis has a global distribution; it causes morbidity and mortality in poultry farming. It is responsible for significant economic losses. Stordeur and Mainil [1] reported that the causative agent, avian pathogenic *E. coli* (APEC), leads to variable syndromes including respiratory tract infections (Airsacculitis) of acute salpingitis and colisepticemia.

In Algeria, avian colibacillosis is responsible for significant economic losses in poultry farming that's leads to appeal for a systematic use of antibiotics.

Antibiotics, like most other veterinary medicinal products are widely used by all producers and especially in poultry farming, without respect of withdrawal period. One consequence is a gradual loss of effectiveness of treatment. For information, at national scale, resistances reached up 88.38% in *E. coli* of avian origin [2]. In the current absence of epidemiological data for monitoring microbial genotypic resistance to commonly used antibiotics, the choice of antibiotic for the treatment remains rather arbitrary.

The present work was conducted to estimate the antimicrobial resistance of *Escherichia coli* isolates from chicken in western Algeria.

2. Materials and methods

2.1 Sampling Site and Procedure: The study was conducted in Western Algeria (Mostaganem, Mascara, Relizane, Chlef, Tiaret and Tissemsilt) during the year 2010. Thus, we collected bacterial strains from individuals (chickens and chicks) with possible colibacillosis, eggs and droppings.

The samples have been directly seeded on agar bromocresol purple (BCP) and incubated for 24 h at 37 °C.

The macroscopic and microscopic identification (bacilli Gram-with peritriche ciliature), the biochemical tests and the analytical profile index 20E were performed according to Quinn [3].

Antibiotic sensitivity was determined by the disc diffusion method on solid Mueller-Hinton medium (Sanofi-Diagnostics Pasteur, France) and performed according to the guidelines of the "Comité de l'Antibiogramme de la Société Française de Microbiologie" [4]). 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), enrofloxacin (5 µg), colistine (10 µg) were laid on the medium. Commercial antibiotic disks were purchased from Bio-Rad (France) and Oxoid (England). 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

3. Results and discussion

In western Algeria, 240 strains of Enterobacteriaceae were isolated according to usual bacteriological procedures. All strains of Enterobacteriaceae isolated had at least one resistance towards these antibiotics. *Escherichia coli* represented 47.5% of these strains (N = 114). The frequency of resistance of each antibiotic tested is shown in Table 1. These frequencies seem high and allow dividing antibiotics into two groups the first group includes antibiotics for which a high level of resistance is noticed: Tetracycline, enrofloxacin, trimethoprim-sulfamethoxazole, Amoxicillin-clavulanic acid and ceftiofur

The second group includes antibiotics to which resistance is low: colistin, chloramphenicol and gentamicin. These results join those reported in Dakar where all strains were resistant to at least one antibiotic [5]. The use of antibiotics could constitute a significant factor in the appearance of emergence selection and dissemination of resistant bacteria in veterinary medicine.

Studies have shown the importance of these resistances in Morocco and Algeria in relation to abusive use of antibiotics [6, 7].

Table1. Antimicrobial resistance of *Escherichia coli* (N = 114)

| Antibiotic (μg) | Resistant strains | |
|------------------------------------|-------------------|--------------------|
| | Number | % (CL 95%)* |
| Tetracycline (30) | 103 | 90.4 (83.4 – 95.1) |
| Enrofloxacin (50) | 79 | 69.3 (60.0 – 77.6) |
| Trimethoprim-sulfamethoxazole (25) | 80 | 70.2 (60.9 – 78.4) |
| Amoxiciline- clavulinic a. (30) | 105 | 92.1 (85.5 – 96.3) |
| Ceftiofur (30) | 71 | 62.3 (52.7 – 71.2) |
| Colistin (10) | 36 | 31.6 (23.2 – 40.9) |
| Chloramphenicol (30) | 12 | 10.5 (5.6 – 17.7) |
| Gentamycine (10) | 2 | 1.8 (0.2 – 6.2) |

Legend: * 95% confidence interval (exact binomial distribution)

The chloramphenicol and the gentamycin are prohibited in veterinary medicine in Algeria. The fact of noticing a low resistance lets suggest an illicit use but however, less frequent.

Compared to other works in the same region, the rate of antimicrobial resistance seems to know a progressive development for some antibiotics. Indeed, for the three antibiotics are tetracycline, enrofloxacin and trimethoprim-sulfaméthazone it passed respectively from 82%, 14% and 42% [7] to 87%, 45% and 70% [8] to reach exceptional rates of 90.35%, 79.82% and 70.17% in 2010 (Table 1). Likewise, the antibiotic resistance to amoxicillin, clavunic acid, ceftiofur and colistin is very high too.

The rate of resistance to tetracycline (90.35%) is higher than that observed in Morocco [9] but it could be that this rate has also increased since 1988.

Resistance to gentamicin, close to that reported previously in the same region [8] and Iran [10] remains at a low level, reflecting its infrequent use in poultry farming in Algeria.

It appears that the isolated strains possess resistance to at least one antibiotic (Table 2).

Table 2. Strains of *E. coli* multiresistant (N = 114)

| Number of antibiotic | % Resistant strains | Number of antibiotic | % Resistant strains |
|----------------------|---------------------|----------------------|---------------------|
| 0 | 0 | 4 | 19.30 |
| 1 | 0.88 | 5 | 38.60 |
| 2 | 5.26 | 6 | 22.80 |
| 3 | 11.40 | 7 | 1.75 |

Thus, it appears that *E. coli* tend to express high resistance to several antibiotics.

In comparison with the work in the same region (7; 8), we also note in this study the increase in multiresistance reaching 19.3%, 38.6% and 22.8% for 4, 5 and 6 respectively antibiotics (Table 2).

The high rate of resistance to most antibiotics and that of multiresistance to 4-6 antibiotics should not surprise considering the high number of available antibiotics in Algeria and the insufficiency in the application of the legislation relating to their use in therapeutic, prophylaxis and as a growth promoter.

4. Conclusion

In conclusion, this exploratory study indicates the existence of alarming rates of individual and multiple antimicrobial resistances concerning *E. coli*. This issue is particularly important because there is a high risk of human contamination following the manual operations relating to the slaughter of poultry. Thus, the increase in resistance to antibiotics can heavily handicap the treatment of the human diseases. Undoubtedly, there is an effort to carry out for a better understanding of the situation of antimicrobial resistance in *E. coli* in western Algeria. The establishment of an epidemiological surveillance network based on genotypic analysis of antibiotic resistance for the main pathogenic bacteria isolated from birds, cattle and sheep sick proves to be a priority in the Maghreb region.

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