ORIGINAL ARTICLE

ANTIBIORESISTANCE OF *ESCHERICHIA COLI* STRAINS ISOLATED FROM TURKEY MEAT MARKETED IN KENITRA CITY (MOROCCO)

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ABSTRACT

Background: The intensive use of veterinary drugs contributes to the emergence of bacterial resistance. Indeed, the most worrying problem is the appearance of multi-resistant bacteria knowing that some strains are resistant to all known antibiotics. **Objective:** The objective of this work is to determine the prevalence of *Escherichia coli* multi-resistance isolated from turkey meat marketed at Kenitra city (Morocco). **Material and methods:** 82 *Escherichia coli* strains were isolated from turkey meat marketed in Kenitra city (Morocco). The resistance to 14 antibiotics was done by the standard antibiogram method with inhibition diameters. **Results:** The antibiogram has revealed a very good sensitivity of strains against four antibiotics: Gentamicin (95.1%), Ampicillin (89%), Colistin (86.5%) and Enrofloxacin (80.4%). However, a mean sensitivity was observed in three antibiotics: Spectinomycin (52.4%) Trimethoprim / Sulfamethoxazole (46.3%) and Ciprofloxacin (42.6%). In addition, the highest percentages of resistance were observed in Erythromycin (97.5%), Tetracycline (95.1%), Norfloxacin (92.6%), Kanamycin (82.9%), Nalidixic acid (79.2%) and Cefalotin (67%). These results reveal a difference in the prevalence of *Escherichia coli* resistance against all antibiotics. **Conclusion:** This study shows that the *Escherichia coli* presented in turkey meat are multi-resistant against all tested antibiotics.
Keywords: *Antibiotic, Public health, residue, Intoxication, multidrug.*

1. INTRODUCTION

During its life, poultry is a subject for various bacterial, viral or parasitic infections. Bacterial infections are widespread in all farms, which require the use of antibiotics to protect and improve the health status of poultry and increase poultry productivity. But, unfortunately, the abusive and uncontrollable use of these drugs does not often obey the rules of antibiotic therapy and has led to the development and emergence of resistant bacteria to antibiotics in both humans and animals. The immediate result of this resistance is the therapeutic failure in breeding thus leading to fatal situations in the treatment of certain serious infections [1].

This situation has generated antimicrobial resistance which has now become a major concern in public health and animal health for some antibiotics. Many studies have found a relationship between the use of antimicrobials and their resistance levels in animals [2]. The typical example of this situation is *Escherichia coli* (*E. coli*), which is the causal agent of several avian diseases. In Morocco, bacterial resistance to antibiotics is constantly evolving and is a major public health problem. Indeed, during the past four decades, bacteria have shown an unlimited ability to escape the action of antimicrobials. As a result, the chances of developing and marketing new antibiotics to the market are reduced. To this excessive use of antibiotics is added the appearance of the risks of residues on human health which can lead to contamination by zoonotic bacteria resistant to medical antibiotics.

The progress of the morphological and metabolic knowledge of bacteria permit to understand antimicrobial resistance mechanisms [3] and to show that the pathogens involved in the propagation of resistance genes are in most cases common to the human and animal [4].

2. MATERIALS AND METHODS

2.1 Study site:

In Morocco, industrial poultry farms are concentrated on the Atlantic coast, particularly in the Kenitra region, because of its favorable climate and proximity to major consumption centers (Casablanca and Rabat). Turkey meat samples were collected from 24 points of sale in Kenitra city from June to September 2016.

2.2 Escherichia coli strains:

The identification of isolated *Escherichia coli* strains was done on a total of 168 analyzed samples. Thus, 82 isolates of Escherichia coli were identified. The identification was carried out with a classical gallery and the confirmation was made by the gallery API 20E.

2.3 Sensitivity to antimicrobials:

In this study, 14 antibiotics were chosen according to their natural spectrum, their authorization in poultry production, their wide use in the treatment of avian colibacillosis in Morocco [5] as well as their representativeness vis-à-vis family [6]. These are: Ciprofloxacin (CIP) 5µg, Norfloxacin (NOR) 10µg, Streptomycin (S) 300µg, Ampicillin (AMP) 25µg, Colistin (CT) 10µg, Erythromycin (E) 15µg, Nalidixic acid (NA) 30µg, Ofloxacin (OFX) 5µg, Kanamycin (K) 30µg, Trimethoprim / Sulfamethoxazole (TMP / SUL) 1.25 / 23.75µg, Tetracycline (TE) 30µg, Gentamicin (GM) 10µg, Cefalotin (CF) 30µg and Enrofloxacin (ENR)) 5µg that was tested because of its high activity on *E. coli* [7].

The bacterial suspension is prepared from viable colonies for 24 to 48 hours, well homogenized in 8.5% NaCl solution. After inoculum preparation, flood inoculation was performed on the entire surface of the nutrient Muller-Hinton agar medium (MH, Oxoid CM0337). From the application of the discs impregnated with specific antibiotics to be tested, the antibiotics diffuse uniformly. After incubation, at 36 ± 1 °C, for 24 hours, the discs are surrounded by circular zones of inhibition.

2.4 Reading and interpretation:

The reference strain *Escherichia coli ATCC 25922* is used for quality control. The antibiograms were read using a vernier caliper to measure the inhibition diameter accurately. They are expressed in millimeters and are interpreted in terms of clinical categories "sensitive", "intermediate" or "resistant" to different antimicrobials, according to the critical points recommended by the CA-SFM. The isolates thus qualified as "intermediate" were counted in the "resistant" category to obtain a dichotomous result taking into account an onset of evolution towards non-sensitivity [8].

3. RESULTS

The results of the study of the sensitivity and resistance of *Escherichia coli* strains to the 14 selected antibiotics are present in Table 1. They show that there is a significant prevalence of antimicrobial resistance among strains isolated from fresh turkey meat.

Table 1: Number of *Escherichia Coli* strains resistant/sensitive for each tested antibiotic.

Antibiotics	Number of resistant and intermediary strains	Number of sensitive strains
CIP	47	35
NOR	76	6
S	39	43
AMP	9	73
CT	11	71
E	80	2
NA	65	19
OFX	51	32
K	68	14
TE	78	4
GM	4	78
ENR	16	66
CF	55	27
TMP/SUL	44	38

CIP: Ciprofloxacin, **NOR:** Norfloxacin, **S**: Streptomycin, **AMP**: Ampicillin, **CT** Colistin, **E**: Erythromycin, **NA** Nalidixic acid, **OFX** Ofloxacin, **K** Kanamycin, **TMP / SUL** Trimethoprim / Sulfamethoxazole, **TE**: Tetracycline, **GM**: Gentamicin, **CF** Cefalotin, **ENR:** Enrofloxacin.

As illustrated in Figure1, the antibiogram revealed a very good sensitivity of the strains against four antibiotics: Gentamicin (95.1%), Ampicillin (89%), Colistin (86.5%) and Enrofloxacin (80.4%). In contrast, a mean sensitivity was observed in three antibiotics: Spectinomycin (52.4%) Trimethoprim / Sulfamethoxazole (46.3%) and Ciprofloxacin

(42.6%). In addition, the highest percentages of resistance were observed in Erythromecleu (97.5%), Tetracycline (95.1%), Norfloxacin (92.6%), Kanamycin (82.9%), Nalidixic acid (79.2%) and cefalotin (67%).

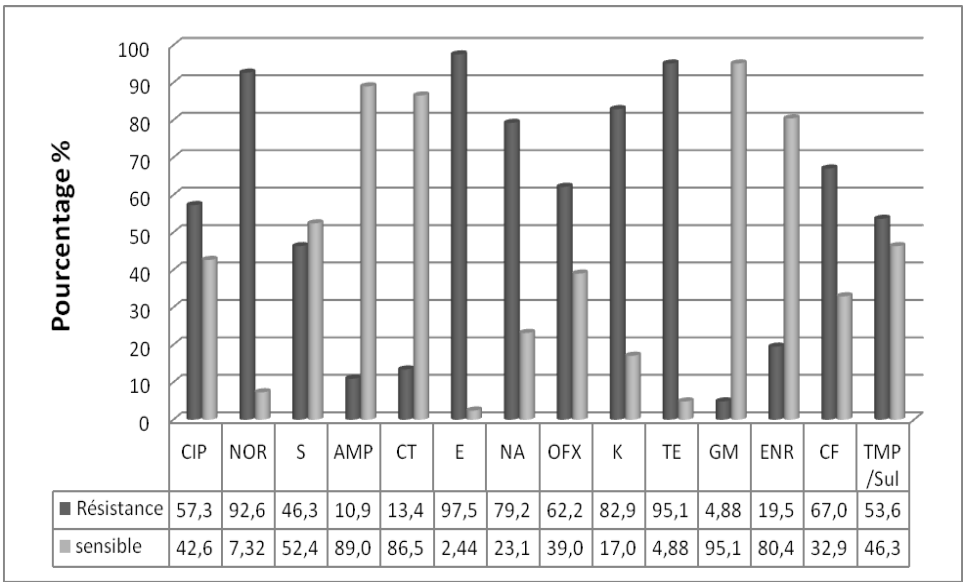

Figure 1: Profile of the sensibility of Escherichia Coli strains against antibiotics.
CIP: Ciprofloxacin, **NOR:** Norfloxacin, **S**: Streptomycin, **AMP**: Ampicillin, **CT** Colistin, **E**: Erythromycin, **NA** Nalidixic acid, **OFX** Ofloxacin, **K** Kanamycin, **TE**: Tetracycline, **GM**: Gentamicin, **ENR:** Enrofloxacin, **CF** Cefalotin, **TMP / SUL** Trimethoprim / Sulfamethoxazole.

All the isolate tested present multi-resistance against many antibiotics (at least three) (Table 2); this situation is catastrophic because 14.63% of strains are resistant to five antibiotics, 23.17% are resistant to six antibiotics and 12.20% are resistant to nine antibiotics tested.

Table 2: Frequency of resistance of *Escherichia coli* strains isolated from turkey meat marketed in Kenitra.

Number of tested antibiotics (among 14)	Number of resistant strains (among 82)	Frequency of resistance (%)
3	4	4,88
4	5	6,10
5	12	14,63
6	19	23,17
7	19	23,17
8	13	15,85
9	10	12,20

4. DISCUSSION

Our results confirm the work of Jouahri (2010), Soufi et al., (2009) and Jouy et al., (2004), who reported high levels of resistance of tetracycline and Trimethoprim/ Sulfamethoxazole in strains of *E. Coli* from poultry meat [9,10,11]. This high percentage reflects the frequency and duration of use of these antibiotics. Thus, tetracycline occupies a predominant place in veterinary provisions, the resistance to this antibiotic is quite widespread [12,13]. It is usually due to a gene plasmid that can be easily acquired by bacteria [14].

The administration of the combination Trimethoprim / Sulfamethoxazole during the start phase of chicks permits to prevent omphalitis [15]. In recent years, this association has been used extensively to control avian colibacillosis and salmonellosis because of its relatively low price and to overcome the resistance developed against tetracyclines and sulfonamides [16]. In fact, 64% of veterinarians surveyed in different regions of the country used this combination to treat colibacillosis [5].

The proportion noted of strains resistant to nalidixic acid (79.27%) was higher than the result reported by El Allaoui et al. (2013), Soufi et al., (2009) and Zoubair et al., (2016) among *E. Coli* of avian origin [17-10-18]. For Ciprofloxacin, the rate is higher than that reported by El Tawab et al., (2015) and lower than the value reported by Akond et al., (2009) [19,20]. These resistances may be due to the use of certain quinolones and fluoroquinolones in the poultry diet and water drinking.

In addition, the sensitivity to Colistin and gentamycin is confirmed by several authors [21,22,23-15]. It constitutes valuable antibiotics in human medicine, and their resistance is relatively low. This low level of resistance reflects the low or moderate use of these two molecules in poultry farming. These antibiotics do not cross the intestinal barrier and are therefore inactive on systemic Colibacillosis. However, they help to control respiratory or intestinal pathogenic Colibacillosis. According to an investigation carried out by El-Youbi over a period of 18 months from July 1, 2012, to December 31, 2013, in eastern Morocco, Enrofloxacin is the preferred antibiotic in avian therapy according to private veterinarians [24]. It is characterized by its bactericidal activity with a low minimum inhibitory concentration (MIC) for many bacteria [25]. It is absorbed very rapidly and widely distributed in the body by reducing the mortality associated with E. coli and mycoplasma [26]. However, its massive use could lead to cross-resistance to other quinolones used in human therapy [27].

The percentage of strains resistant to 2, 3 and 4 antibiotics is lower than the values founded by Van Den Bogaard (2001), Aggad (2010), and Rahmatallah (2017) [28,29-15]. The percentage of resistant strains to 5, 6 and 9 antibiotics is higher than the values founded by Zhao (2005) [30].

The high rates of multi-resistance may be due to the fact that all strains come from offending (firm) media where the antibiotics are in daily use. Indeed, Novick (1981) and Nowroozi et al., (2004) have shown that the uncontrolled use of antibiotics in poultry production lead to an increase of the emergence of multi-resistant bacteria [31,32]. In order to limit the selection and multiplication of multidrug-resistant strains in the European Union, the European Commission for "regulation" has banned the use of antibiotics as a means of stimulating growth inbreeding [33].

The significant increase of antibiotic resistance incidence in *E. coli* strains (isolated from marketed turkey meat) is probably due to several factors such as: the increasing use of antibiotics as a growth factor, prevention of disease, inappropriate use of antibiotics to treat certain diseases, as well as the transfer of resistance between different bacteria. Also, there is a possibility of cross-resistance between antibiotics used in poultry.

5. CONCLUSION

This study showed high levels of *E. coli* resistance against several antibiotics especially for the following molecules; Erythromycin, Tetracycline, Norfloxacin, Kanamycin and nalidixic Acid. The excessive and uncontrolled use of these antibiotics for the prevention and treatment of avian diseases would be the main cause of selection of resistant E. coli strains. Extension and technical support actions need to be strengthened among pastoralists to improve technical management of breeding to avoid diseases caused by poor husbandry practices and, consequently, reduce the use of antibiotics. Care should also be taken to ensure the proper use of antibiotics in veterinary medicine. The prescription of this particular class of drugs should only be done on scientific grounds (antibiograms) to provide well-reasoned and targeted treatments in each case. The presence of multi-resistant strains should alert health officials in the country to establish surveillance programs to control the antimicrobial resistance of pathogenic bacteria.

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