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Antibiotic Resistance of *Escherichia coli* Strains Isolated from Broiler Meat in Morocco

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ABSTRACT

The spread of multidrug-resistant (MDR) enterobacteria worldwide is a major public health threat. Resistant to at least three classes of antibiotics, MDR enterobacteria cause infections that escape first-line treatment. *Escherichia coli* is the most frequently isolated bacterium in poultry industry. We followed the pattern and trend of antibiotic resistance of strains of *E. coli* isolated from poultry meat destined for human consumption sold in Morocco during the period 2016-2018, and thus detected strains that would be multidrug-resistant (MDR). In this study we investigated the resistance of 240 strains of *E. coli* isolated from poultry meat to 13 antibiotics by using the agar diffusion susceptibility test method. Results showed high resistance to ciprofloxacins (87.5%), tetracycline (75%), trimethomprime-sulfametoxazole (3rd generation) (70.8%), nalidixic acid (62.5%) and cefotaxime (50%), ampicillin (45.8%); and we detected low resistance to gentamicin (29.1%). In total, 50 isolated strains of *E. coli* (20.8%) have shown MDR. These results are useful to practicing veterinarians trying to avoid therapeutic failures and constitute an important database for pharmacovigilance and epidemiological surveillance of antimicrobial resistance in the country.

Key words: *Escherichia coli*, Poultry meat, Antibiotic, Multidrug-resistant.

INTRODUCTION

The pressure exhibited by the misuse of antibiotics induces the emergence of multidrug-resistant bacterial strains, which cause a major and growing problem in public health. Indeed, the use of antibiotics in the livestock sector is increasing to such an extent that it can lead to negative aspects for human health, animal health and the environment (Ellinger, 2019). The poultry sector plays an important role in the Moroccan economy. It oscillates between a modern intensive production and traditional agricultural activity and generates more than 23.2 billion Moroccan dirhams MAD (2.8 billion US dollars). Nowadays, this sector provides 400.000 direct and indirect jobs, including marketing and distribution channels (Sifou *et al.*, 2016). The intensive use of antibiotics for therapeutic, preventive or growth-promoting purposes makes poultry farms a privileged place for the reappearance, development, and spread of resistant pathogens.

Poultry and its products are considered to be the main vector of pathogenic bacteria, such as *Salmonella serovars*, *E. coli*, and *Klebsiella*s pp, which cause foodborne infections in humans. Avian diseases is an inclusive term designating a large group of acute and chronic diseases of poultry caused by any one genus or more member of the family *Enterobacteriaceae* (Soliman *et al*., 2020).The prevalence of *E. coli* highly resistant to antibiotic has been recorded more frequently in poultry meat than in all other types of meat (Amara *et al*., 1995). Extended spectrum β-lactamases are plasmid-encoded enzymes found in Gram-negative bacteria, particularly in Enterobacteriaceae, conferring resistance to cephalosporins of the first, second, and third-generations, while they are inhibited by clavulanic acid.

In Morocco, previous surveys reported a very high rate of bacterial resistance; 45.5% of multi-resistant *Klebsiella spp.*, 50% of methicillin-resistant *Staphylococcus* spp., while a resistance of 31% was recorded in E. coli toward fluoroquinolones (Amara *et*

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al., 1995, Rahmatallah *et al*., 2017). Indeed, E. coli was the most frequently isolated bacterium in these farms. It is developing resistance tocertain antibiotics, and in response to this situation, several countries have developed networks to monitor the resistance of bacteria to antibiotics (Hussain *et al*., 2017). The present study aims to investigate the resistance of 240 strains of *E. coli*, isolated from samples of broiler meat during 2016-2018, to thirteen (13) antibiotics that are currently used in the poultry industry and in human antibiotherapy.

MATERIALS AND METHODS

E. coli strains

The present study was carried-out on a total of 240 strains of *E. coli* isolated from samples of broiler meat intended for human consumption and sold in regions throughout Morocco during the period 2016-2018. Studied strains were stored in the Microbiology and Food Hygiene Laboratory of the National Institute of Hygiene (NIH) and the Microbiology Laboratory of the High School of Technology (HST). The cultures of *E. coli* were performed in EMB medium (eosinemethylene blue) after incubation at 37°C for 18 to 24 hours. Colonies with a metallic green or fluorescent reflection on EMB have undergone biochemical identification tests by the usual galleries, mini-galleries API 20 E and ID 32 E **(**Barnes *et al*., 2003). These biochemical tests are based on the capacity of bacteria to hydrolyze some hydrocarbons such as glucose, lactose, and mannitol **(**Singleton, 2004). Tests of antibiotic susceptibility were performed using the standard agar disk diffusion as described by Bauer *et al.* (1966) and recommended by Matuschek *et al.* (2014), and CASFM/EUCAST (2018). The medium used is Mueller-Hinton agar (MH).

Antibioresistance test

The thirteen antibiotics tested were chosen in order to establish comparisons between the main families of antibiotics available on the Moroccan market and used in veterinary medicine and in the surveillance of antibiotic resistance worldwide: ampicillin (AMP : 10 µg) amoxicillin + clavulanic acid (AMC : 20/10 µg), ceftriaxone (CRO : 30 µg), cefotaxime (CTX : 30 µg), imipenem (IMP : 10 µg), tetracycline (TET : 30 µg), nalidixicacid (NAL : 30 µg), ciprofloxacin (CIP : 5 µg), norfloxacin (NOR : 10 µg), trimethoprim + sulfametoxazole (SXT : 25 µg), trimethoprim (TMP : 5 µg), chloramphenicol (CHL : 30 µg) and gentamicin (GEN : 10 µg) (Table 1).

The selected cultures had a metallic green reflection on EMB agar medium. The dominant biochemical profile was glucose, lactose, mannitol, indole and gas tests, positive and citrate, H_2S and urease tests, negative. The use of the API 20E mini-galleries made it possible to identify the 240 *E. coli* strains studied.

After incubation, the diameters of the inhibition zones were measured. Then the bacterium was classified either as sensitive or resistant. According to the practical manual of the antibiogram used at the INH, any intermediate strain is considered a sensitive strain. The quality control was performed with the *E. coli* strain ATCC 25922, which was treated under the same conditions as the strain to be tested; this test validates the test result and makes it possible to control the antibiotic discs and the quality of the MH medium.

RESULTS

The antimicrobial resistance profile of the studied strains is quite diverse, ranging from wild-type to multi-resistance profiles. The 240 *E. coli* strains isolated and the 2 strains of quality control were analyzed to study their resistance profile against the 13 selected antibiotics. According to the Moroccan standard (NM), a multidrug-resistant strain is any strain resistant to more than 3 antibiotics. The results reported show that among the 240 *E. coli* studied strains, 230 strains (95.8%) showed resistance to at least one (1) antibiotic. However, 60 strains (25%) showed resistance to five (5) antibiotics. Moreover, 50 strains (20.8%) were resistant to eleven (11) antibiotics (Table 2). Finally, 10 strains (4.1%) among the 240 studied were found to be sensitive to all antibiotics.

The antibiotics with the highest levels of resistance are ciprofloxacin with 87.5%, tetracycline 75%, trimethomprime-sulfametoxazole (70.8%), nalidixic acid (62.5%), cefotaxime (3rd generation) (50%), ampicillin (45.8%), and low resistance to gentamicin (29.1%).

Table 1: Concentrations of 13 antibiotics tested on 240 isolated *E. coli* strains

Antibiotic group	Antibiotics (µg)	Abbreviations
	Ampicillin (10)	AMP
β-lactam	Amoxicillin+clavulanic acid (20/10)	AMC
	Ceftriaxon (30)	CRO
	Cefotaxim (30)	CTX
	Imipenem (10)	IMP
Tetracycline	Tetracyclin (30)	TET
	Nalidixic acid (30)	NAL
Quinolone	Ciprofloxacin (5)	CIP
	Norfloxacin (10)	NOR
Sulfonamide	Trimetoprim+ sulfametoxazole 25 (1.25+23.75)	SXT
Diaminopyrimidines	Trimethoprim (5)	TMP
Chloramphenicol	Chloramphenicol (30)	CHL
Aminoglycoside	Gentamicin (10)	GEN

Table2: The percentage of resistance and sensitivity of tested *E. coli* to each antibiotic

Antibiotics	Resistance (%)
Ampicillin (AMP)	45.8
Amoxillin+ clavulanique acid (CA)	45.8
Ceftriaxon (CRO)	16.6
Cefotaxim (CTX)	50
Imipenem (IMP)	4.7
Tetracyclin(TET)	75
Nalidixic acid (NA)	62.5
Ciprofloxacin (CIP)	87.5
Norfloxacin (NOR)	66.6
Trimetoprim + Sulfametoxazole (SXT)	70.8
Trimethoprim (TMP)	33.3
Chloramphenicol (C)	33.3
Gentamicin (GEN)	29.1

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Table 3: The frequencies of the multi-resistance types recorded in the different strains of *E. coli*

Antibio-types	Frequency %
CTX; TET; CIP	8.3
CTX; SXT; CIP; NOR; TET	25
CTX; NA; SXT; CIP; NOR; TET	8.3
AMC; NA; GEN; AMP; CIP; TET; TMP	4.1
AMC; CTX; NA; SXT; AMP; CIP; NOR; TET	8.3
AMC; CTX; NA; SXT; AMP; C; CIP; NOR; TET	4.1
AMC; NA; SXT; GEN; AMP; C; CIP; NOR; TET; TMP	4.1
AMC; CTX; NA; SXT; GEN; AMP; C; CIP; NOR; TET; TMP	20.8

Table 4: Comparison of resistance rates in 6 different studies conducted in Morocco between 1995 and 2018.

Antibiotics	This study (%)	Rahmatallah *et al.*, 2017 (%)	Hafed *et al*., 2015 (%)	El houadfi and Zekhnini 2009 (%)	Jaouzi *et al.*, 2004 (%)	Amara *et al*., 1995 (%)
Amoxicillin	88.2	90	88.2	72	65	-
Ampicillin	45.8		-	96	-	14
Chloramphenicol	75	-	-	20	65	41
Florfenicol	-	61.5				
Enrofloxacin	Ciprofloxacine 76.3	75.9	76.3	20	34	23
Oxytetracyclin	Tétracycline 69.2	100	97	96	90	65
TMP-S	65.3	82.2	-	-	68	61
Colistin	12.5	2.9	12.5	20	13	0.4
Gentamicin	29.1	24.7	-	-	43	7
Fosfomicyn	-	16.1	-	-	-	-

The resistance profiles of *E. coli* strains isolated have been identified. As shown, the resistance was observed for all antibiotics. Multidrug resistance (resistance to more than 3 antibiotics) was found in 83.3% of *E. coli* strains. Indeed, 50 *E. coli* strains were found to be MDR (20.8%): resistant to both ampicillin, amoxicillin + clavulanic acid, cefotaxime, tetracycline, nalidixic acid, ciprofloxacin, norfloxacin, trimethoprim + sulfamethoxazole, trimethoprim, chloramphenicol, gentamicin, which are all antibiotics of choice for treatment. Thus, multidrug resistance can involve up to 11 antibiotics based on the observed resistance patterns (Table 3).

Table 4 compares the resistance rates recorded in Morocco since 1988. Indeed, we observed that *E. coli* resistance to ampicillin increased from 14% in 1995 to 96% in 2009, while amoxicillin was not used in poultry during the 1995 study. The resistance rate to amoxicillin increased from 65% in 1999 to 72% in 2009, then to 88.2% in the present study. Rahmatallah *et al*. (2017) showed an almost similar 90.1% resistance to amoxicillin. As for enrofloxacin, its resistance rate was 23% in 1995, and then went up to 34% in 1999, and to 72% in 2003, and finally reached 75.9% in our study. The rate of resistance to oxytetracycline was already high in 1988 (82%) but reached a maximum of 100% in 2015. The resistance test to tetracycline gave a rate of 75 % in our study. This situation is very worrying since in other countries, such as France, resistance to this antibiotic is only 50% **(**Resapath, 2014).

The resistance rate to chloramphenicol (C) increased significantly, while the resistance to colistin remained low and practically unchanged. As for the resistance rate to gentamicin, it remained close to the results of Rahmatallah *et al.* (2017) in Morocco, but high compared to those recorded in France during the same year.

DISCUSSION

Antibiograms carried out on *E. coli* strains revealed high levels of quinolone resistance and fluoroquinolones, including nalidixic acid (100%). Indeed, other studies have showed that the reduced sensitivity to nalidixic acid is more and more common in many parts of the world due to the massive use of this antibiotic (Kmet and Kmeová, 2010, Mekaiel *et al*., 2017, Chung *et al*., 2017). Moreover, our study has shown that 62.50% of isolated *E. coli* strains are resistant to nalidixic acid. This resistance is related to the use of this antibiotic in the treatment of poultry diseases (Caruso *et al*., 2018). Moreover, a high resistance (87.5%) of *E. coli* to ciprofloxacin was found. Our results are higher than those obtained by Rahmatallah *et al.* (2017) in the same region (75.9%). Concerning the cyclins, especially tetracycline, a higher resistance (75%) was reported in several studies (Filali *et al*., 1988). This resistance is due to the systemic use of this class of antibiotics in industrial poultry farming as a growth promoter. *E. coli* strains resistant to trimethoprim-sulfamethoxazol represent a percentage of 70.8%, while Halfaoui *et al.* (2017) reporteda percentage of 88.9%. Resistance to gentamicin and chloramphenicol was 29.1% and 33.3%, respectively, while Hafed *et al.* (2015) showed a close resistance to gentamicin (24%), whereas Rahmatallah *et al*. (2017) found a high resistance rate (61.5%) for the florfenicol, which is an analogue of chloramphenicol.

However, a moderate rate of strains that presented resistance to ampicillin and amoxicillin-clavulanic acid showed an equal level of resistance (45.83%) compared with 68.62% according to the study by Hafed *et al.*(2015)**.** This is due to the fact that this antibiotic was originally used in first-line antibiotic therapy and has then lost its effectiveness following the appearance of resistance mechanisms in *E. coli*.

Results from our study showed that 83.3% of the strains are multi-drug resistant. These results are lower than those published by Subedi *et al.* (2018)**,** who found that 94% of strains are resistant to three or more of the antibiotics tested. In this study, the results observed in the isolates demonstrate that the resistance trend of *E. coli* to some antibiotics is increasing in Morocco.

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In comparison with results reported by the French surveillance network of antibiotic resistance (Resapath, 2014) for the years 2010 to 2014, the resistance rates recorded in Morocco is very high. It is important to clarify that the efforts made in France to reduce antibiotic consumption have given encouraging results (tetracycline had a resistance frequency of 84% in 2010, and this rate was reduced to 50% in 2014). The levels of resistance against certain antibiotics are tending to decrease in France, while high rates are still observed in Morocco. The frequencies of resistance to florfenicol are 1% in France (2014) against 62% in Morocco (2014). Moreover, the resistance to chloranmphenicol found in our study is 75%. These differences also observed for resistance to enrofloxacin which was 6% in France (2014) against 76% in Morocco (Rahmatallah *et al*, 2017). These examples demonstrate the extent of antimicrobial resistance risk in Morocco. The percentage of multidrug-resistant strains could be explained by repeated exposure of animals to these antibiotics, but also probably by the excessive and uncontrolled taking of antibiotics by the general population without medical consultation, and also by the direct sale of these drugs in pharmacies without medical prescription. Indeed, some of these antibiotics are incorporated into animal feeds as a preventive measure or as food additives for various purposes, in particular for increasing muscle mass (Rasheed *et al*., 2014)**.** According to EFSA and ECDC reports (EFSA-ECDC, 2014), *E. coli* is an excellent indicator of the level of resistance of enterobacteria in breeding animals, because it is widespread in agricultural environments.

The frequencies of resistance of E. *coli* to the antibiotics found in our study are very worrying. Moreover, the high tendency of *E. coli* to show multi-drug resistance aggravates the current situation. This study highlights the high prevalence of *E. coli* resistant to different antibiotics in broilers for human consumption in the Rabat-Salé-Kénitra area. This resistance can be transferred from animals to humans not only through the food supply, but also after contact with these animals and their excrement, increasing the risk of treatment failure when these critical antimicrobial classes are used in human patients. Also, the danger of transmitting antibiotic resistance between multidrug-resistant strains and strains that are either non-resistant or resistant to one or more antibiotics must be emphasized. Further studies are needed to cover more areas of the country, more classes of antibiotics used, and to determine the distribution of serotypes of *E. coli* multidrug-resistant strains. This will provide a good illustration of the problem at the national level, as well as the necessary evidence for decision-making on this issue.

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