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Full Length Research Paper

Resistance to 3rd generation cephalosporin of *Escherichia coli* isolated from the feces of healthy broilers chickens in Algeria

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High resistance of *Escherichia coli* have been demonstrated to 3rd generation cephalosporin in livestock, especially in broiler chickens; however, data on emission sources of these bacteria into Algeria are still rare. From January to March 2014, a preliminary epidemiological study of *E. coli* contamination in healthy broiler chicken flocks was carried out in the regions of Tlemcen, Algeria. 21 *E. coli* resistant isolates were examined to 3rd generation cephalosporin antibiotics (ceftriaxone, ceftazidime and cefotaxime). The antimicrobial susceptibility was determined by disk diffusion, and the MICs were determined by agar dilution method with Antibiogram Committee of the French Society for Microbiology (CA-SFM) 2013 guidelines. All strains were resistant to ceftriaxone and cefotaxime referring to CA-SFM, Antibiogram Committee of the French Society for Microbiology (EUCAST), and Clinical and Laboratory Standards Institute (CLSI). However, the resistance rate of ceftazidime is different according to the breakpoints criteria used; the susceptibility result of CA-SFM and EUCAST is similar for each farm. Farm B 50% of *E. coli* was resistant and 50% was susceptible, and 21% was susceptible and 79% was resistant for the farm C. However, comparing these two with CLSI, all strains were susceptible to ceftazidime.

Key words: *Escherichia coli*, antimicrobial resistance, 3rd generation cephalosporin, feces, broilers chicken.

INTRODUCTION

A large number of antimicrobial and anticoccidial agents are used in modern food animal production including broiler production resulting in the emergence of antimicrobial resistance, which is a cause of concern worldwide (Aarestrup et al., 2008; Pangasa et al., 2007). In recent years, antimicrobial resistance and especially multi-drug resistance, has become very common in clinical isolates, including *Escherichia coli* isolates of animal origin (Dolejská et al., 2008). *E. coli* strains are a part of intestinal normal microflora of many animals, including humans and birds (Brzuszkiewicz et al., 2001). *E. coli* is considered to be an excellent indicator of antimicrobial resistance for a wide range of bacteria (Bogaard and Stobberingh, 2000; McEwen and Fedorka-

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Cray, 2002). Resistant *E. coli* can be transmitted to humans from animals. A large proportion of resistant isolates causing human infections are derived from food animals (Jakobsen et al., 2010).

Collingnon et al. (2013) have extrapolated values from all over Europe they have found if 56% of the 3rd generation cephalosporin-resistant *E. coli* (G3CREC) were derived from poultry. In addition, Depoorter et al. (2012) showed that acquired resistance of *E. coli* to 3rd generation cephalosporin antimicrobials is a relevant issue in intensive broiler farming. G3CREC can be transferred from broiler to humans, not only through direct contact but also indirectly. This indirect transfer involves mainly consumption of broiler meat or contact with surface water or vegetables contaminated with broiler excreta (Blake et al., 2003).

In Algeria, since the 1980s, the emergence of the poultry industries increased the consumption of animal proteins at a much affordable cost (Ferrah et al., 2003). The recent study of Aboun (2012) showed that from 989 *E. coli* isolated from four veterinary laboratories, the percentages of extended-spectrum beta-lactamases (ESBL) production were 1.3, 20 and 85% in three laboratories: Pasteur Institute of Algeria, Constantine Regional Veterinary Laboratory and the Regional Veterinary Laboratory Laghouat, respectively. The antibiotics β-lactams used in Algeria are ampicillin, amoxicillin, oxacillin, penicillin, amoxicillin-clavulanate, cephalothin and ceftiofur. Growth factors antibiotics are not incorporated into animal feed and are banned from use since April 2007 (Kechih-Bounar).

Our objectives of this study were to estimate the frequency of resistant *E. coli* in feces samples of healthy broiler chickens in the regions of Tlemcen, during the rearing of broilers period and to identify the antimicrobial resistance of isolates to 3rd generation cephalosporin antibiotics and compared to CA-SFM 2013 and new version Antibiogram Committee of the French Society for Microbiology (CA-SFM) 2014, European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2014 and Clinical and Laboratory Standards Institute (CLSI) 2014 breakpoints criteria.

MATERIALS AND METHODS

Study area

Tlemcen is a town in Northwestern Algeria and the capital of the province (Wilaya) of Tlemcen (Figure 1). It is open to all midfield: Mediterranean sea in the north; Morocco frontiers in the west, a land place of international exchanges.

This study was conducted in farms around a radius of 40 km from the city of Tlemcen. Each farm is situated in a distance to another.

Sampling

All materials needed for sampling were prepared before leaving the laboratory: a cooler filled with ice, latex gloves, spatula, lighter, sterile tubes, labels, boots, and blouse. For the samples, latex gloves were used to prevent direct contact with the samples; the tubes were marked and labeled before taking the samples not be mixed. For each broiler flocks, the maximum number of samples is 60 feces during the rearing of broilers for 50 to 55 days. Each sample of fresh feces (approximately 10 to 15 g) of broilers was randomly collected soil along sheds (one in each 2 m, and this ensures that fecal samples are representative of the group) with a sterile spatula before soaring with a lighter, and is placed in a sterile tube (60 ml). The tubes were then placed in a cooler, returned to the laboratory within two hours, and analyzed for 6 h after collection.

Isolation/Identification of *E. coli*

Approximately 1 g of each fecal sample was inoculated into tubes, containing 9 ml vice Brain Heart Infusion Broth (Fluka BioChemika, Spain), and incubated aerobically at 37°C for 18 to 24 h without shaking. Platinum loopful of the broth was subcultured on MacConkey agar medium (Fluka BioChemika, Spain) supplemented with cefotaxime 2 mg/ml and incubated aerobically for 18 to 24 h at 37°C. A single colony morphology typical *E. coli* large pink to red was selected and identified by conventional biochemical methods, the Tryptic Sugar Iron Agar, the Oxidase test and API 20E test (bioMérieux, Marcy-l'Étoile, France). One isolate per feces per broiler chicken was accepted. Isolates of *E. coli* were retained between 2 and 6°C.

Antimicrobial susceptibility testing

The minimum inhibitory concentrations (MICs) were determined by agar dilution method in Mueller-Hinton medium (Fluka BioChemika, Spain), in accordance with the Comité de l'Antibiogramme de la Société Française de Microbiologie (CA-SFM) 2013 guidelines. The method of serial dilution in a solid medium was used to determine the bacterial susceptibility to antibiotics. It consists in a standardized bacterial inoculums contact with increasing concentrations of antibiotics. *E. coli* strain ATCC 25922 was used as a control.

RESULT

E. coli in tested poultry farms

From January to March 2014, 160 feces samples were collected from housing of healthy broiler chicken flocks at 4 different rearing sites (farms A, B, C and D) in Wilaya of Tlemcen (Table 1) during fattening in the rearing period for each farm. Each of the four flocks comprised between 800 and 4000 birds per house. The specimens were collected by walking through the housing. Overall 21 non-duplicate strains of *E. coli* were isolated from two farms. The frequency of contamination for the period of study is 13.12% (21/160).

Antibiotic susceptibility of isolated strains

The CA-SFM 2013 is based on three clinical categories which was selected for the interpretation of *in vitro* sensitivity: Sensitive (S), Resistant (R) and Intermediate

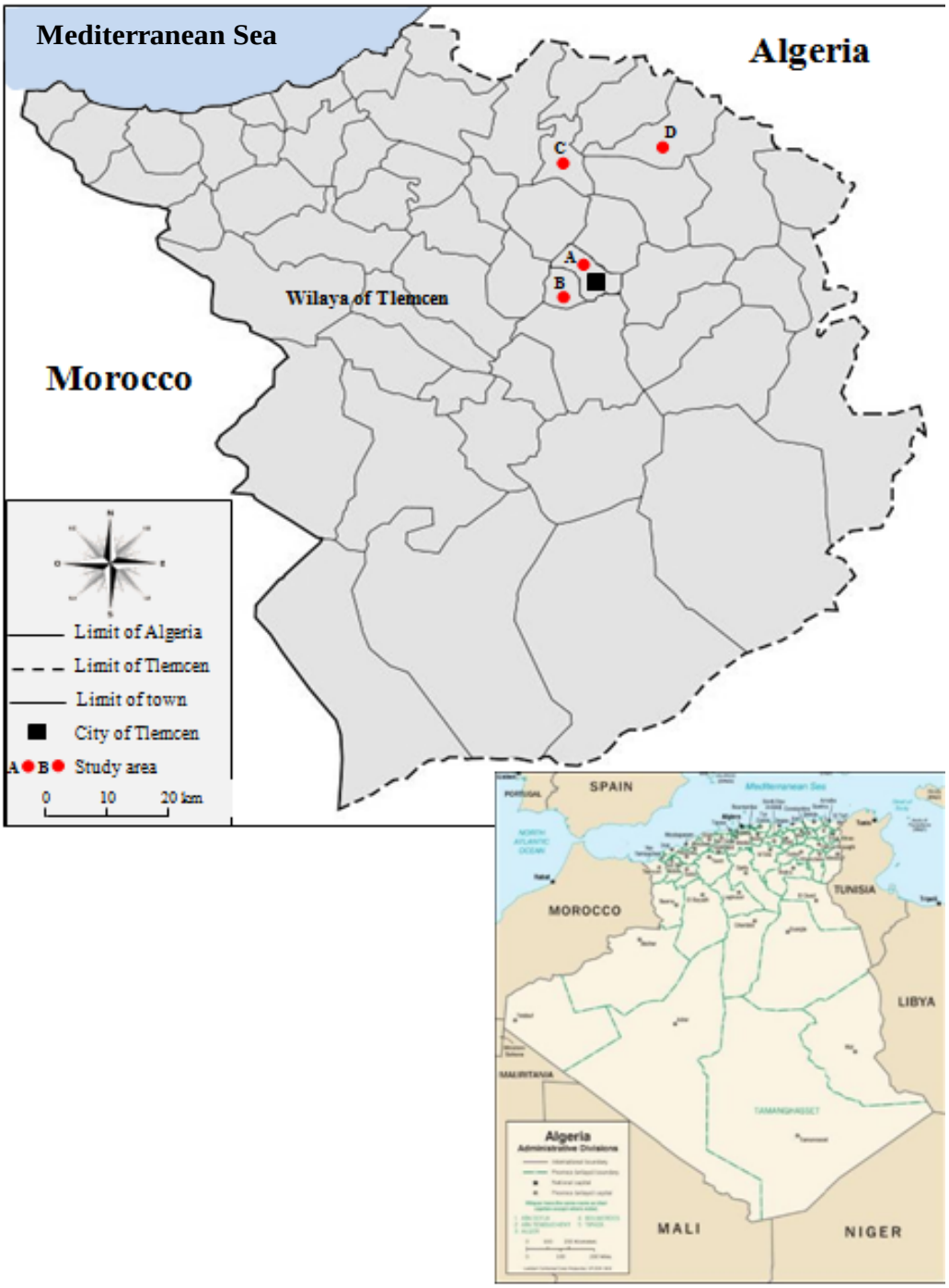

Figure 1. Map of the Wilaya of Tlemcen. Areas shown in circles represent locations of broilers farming activities.

(I). However, in this study the isolates were classified as susceptible (S) or resistant (R) using the zone diameter interpretative standards recommended by CA-SFM 2013. Isolates with intermediate susceptibility were considered susceptible. The results were categorized as very high rate of resistance (>75% of isolates resistant); high rate (>50 to 75%); moderate rate (>30 to 50%); low rate (>10 to 30%), and very low resistance rate (0 to 10%) (Knezevic and Petrovic, 2008).

Table 2 shows that all *E. coli* strains were resistant to ceftriaxone and cefotaxime referring to CA-SFM 2013. The same result observed using CA-SFM 2014, EUCAST

Table 1. Distribution of *E. coli* isolates per farms.

Farm	Number of broilers	Number of samples	Number of *E. coli* isolates
Farm A	4000	40	00
Farm B	4000	60	02
Farm C	4000	20	19
Farm D	800	40	00
Total 04 farms	12800	160	21

Table 2. Susceptibilities rate of isolates of *E. coli* to ceftazidime, ceftriaxone and cefotaxime using CA-SFM 2013 and CA-SFM 2014, EUCAST table v 4.0 and CLSI M100-S24 breakpoint criteria.

Farm	Ceftazidime (CAZ)					Cefotaxime (CTX)			Ceftriaxone (CXM)		
	CA-SFM		EUCAST		CLSI	CA-SFM	EUCAST	CLSI	CA-SFM	EUCAST	CLSI
	S	R	S	R	S	R	R	R	R	R	R
Farm B	50	50	50	50	100	100	100	100	100	100	100
Farm C	21	79	21	79	100	100	100	100	100	100	100

S: Susceptible; R: Resistant.

2014, and CLSI 2014 breakpoints criteria. However, the ceftazidime indicate the resistance of some strains. Comparing the resistance rate of ceftazidime, the susceptibility result of CA-SFM, and EUCAST is similar for each farm; for farm B 50% (*n*=1), *E. coli* was resistant and 50% (*n*=1) was susceptible, and 21% (*n*=4) of *E. coli* was susceptible and 79% (*n*=15) was resistant for farm C. However, comparing these two with CLSI, it was noted that there was great difference; all strains were susceptible to ceftazidime.

DISCUSSION

E. coli in tested poultry farms

To our knowledge, this is the first epidemiological study of 3rd generation cephalosporin-resistant *E. coli* in broiler chicken flocks ever carried out in the Tlemcen region of Algeria. Prior to this research, very little information was available on G3CREC in Algerian poultry farms. The Algerian researchers are much more interested in the study of *Salmonella* (Bouzidi et al., 2012) and the study of *Campylobacter* (Messad et al., 2014).

The presence of cephalosporin-resistant *E. coli* in the intestinal tract of food-producing animals is extensively described in many reports (Hasman et al., 2005; Kojima et al., 2005; Riano et al., 2006; Cloeckaert et al., 2007; Liu et al., 2007). The result of frequency contamination is in accordance even with the rates detected until recently in some countries. Low levels of resistance to cefotaxime, ceftazidime, and cephalothin were observed throughout the study period (18.0 to 27.2%), probably because these antimicrobials are prohibited from use as veterinary products in China (Xiang et al., 2014). Another studies conducted in other countries reported similar results in USA (Tadesse et al., 2012) and in Spain (Blanco et al., 1997). In Belgium, about 35% of the *E. coli* strains isolated from live broilers are resistant to 3rd generation cephalosporins, while over 60% of the broilers are found to be carrier of these 3rd generation cephalosporin resistant *E. coli* after selective isolation (Depoorter et al., 2012).

In this study, two farms were contaminated. A low rate observed in farm B 3.33% (2/60), however, in farm C, a high rate 95% (19/20) and 0% in two farms: A and D.

Antimicrobial resistance

MIC results were interpreted following four sets of guidelines: those published in 2013 and 2014 by the CA-SFM, those published in 2014 by EUCAST, and the CLSI guidelines published in 2014.

The CA-SFM 2013 and new version 2014 susceptibility breakpoints for ceftriaxone, ceftazidime and cefotaxime were ≤1 µg/ml and resistance breakpoints were >2, >4 and >2 µg/ml, respectively. EUCAST 2014 susceptible breakpoints for cefotaxime, ceftazidime and ceftriaxone were ≤1 µg/ml and resistance breakpoints were as CA-SFM. CLSI 2014 susceptibility breakpoints were ≤1, ≤4 and ≤1 µg/ml and resistance breakpoints were >4, >16 and >4 µg/ml, respectively.

There was no difference of resistance rate to these antimicrobials (cefotaxime and ceftriaxone) when compared. For example, the resistance rate of all isolate was 100% to cefotaxime when determined using CA-SFM 2013 and 2014, EUCAST 2014 and CLSI 2014 criteria. However, the susceptibilities of ceftazidime were irrespective of the breakpoints used. In farm B, the

isolated *E. coli* were 50% susceptible to ceftazidime for CA-SFM 2013 and 2014 and EUCAST 2014; and 100% for CLSI 2014. For farm C, the strains susceptible to this antibiotic were 21% for CA-SFM 2013 and 2014 and EUCAST 2014; and 100% susceptible for CLSI 2014.

Compared with CA-SFM 2013 and 2014, EUCAST 2014 breakpoints successfully designated a larger number of isolates as cephalosporin-resistant. Furthermore, CA-SFM 2013 susceptibilities were similar to those using CA-SFM 2014 and EUCAST 2014 guidelines. However, CLSI 2014 susceptibility is very different to CA-SFM and EUCAST. The difference of percentage noted between the two farms return to the numbers of strains isolated from each farm.

CLSI breakpoints may differ from those approved by various regulatory authorities for many reasons, including the following: different databases, differences in interpretation of data, differences in doses used in different parts of the world, and public health policies. Differences also exist because CLSI proactively evaluates the need for changing breakpoints (CLSI, 2014).

The result in this study is in accordance with that released in Spain in 2003, which reported 10.1% cefotaxime resistant *E. coli* isolated from poultry. These levels were gradually rising to 23 and 30% between 2005 and 2008 and they have decreased to 20.8% in 2011. Same dynamics were observed in the Netherlands, with increasing numbers of resistance to 3rd generation cephalosporins from 14.1 to 17.5% between 2005 and 2009 and a decrease to 8% in 2011 (Garcia-Migura et al., 2014).

Conclusion

The data of this survey indicate that *E. coli* isolated from healthy broiler chickens in some farms were resistant to 3rd generation cephalosporin, especially ceftriaxone and cefotaxime, which originated from feces during the rearing of broilers 50 to 55 days. The ceftazidime remains effective for some strains. The result mentions the utility to choose a good interpretation of the results of antimicrobial susceptibility and MICs according the guidelines CA-SFM, EUCAST and CLSI.

The widespread resistance of *E. coli* isolates should raise concerns about imprudent use of antibiotics in veterinary medicine. These observed differences should be further investigated in new prevalence studies.

Conflict of Interest

The authors have not declared any conflict of interest.

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