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## Antimicrobial susceptibility of *Escherichia coli* O157 strains isolated on sheep carcasses in Algéria

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### ABSTRACT

This study aims to investigate the antibiotic resistance of *Escherichia coli* O157 strains, producing Shiga toxins isolated from carcasse surface's of one hundred and fifty (n=151) sheep. Indeed, there is a few data on the epidemiology of STEC O157 strains in Algeria. Thirteen strains of *E.coli* O157:H7 were isolated from 11 sheep carcasses (7.26%). The study of the sensitivity was tested with 31 antibiotics belonging to different classes, the disk diffusion method agar Mueller Hinton (CM337, Oxoid) using NCCLS standards (National Committee for Clinical Laboratory Standards) recommended by WHO was used. The results showed that the three strains carrying the genes (*eae*, *stx1*, *stx2*) are resistant to tetracycline, a strain with (*eae*, *stx2*) genes to furans and a strain with (*eae*, *stx2*) genes, to furans and tetracycline. Eight strains were susceptible to all antibiotics tested. The presence of *E. coli* pathogens strains resistant to antibiotic results on a double public health problem. An extensive knowledge of STEC strains circulating and the introduction of monitoring plans to the various links in the food chain should be conducted at both veterinary and human level.

**Keywords:** *Escherichia coli* O157, Shiga toxin producers, antibiotic resistance, sheep carcasses, Algiers (Algeria).

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### INTRODUCTION

*Escherichia coli* producing Shiga toxin are not only responsible of foodborne poisoning that induced diarrhea and hemorrhagic colitis but also of more severe syndromes for man as hemolytic uremic syndrome that can cause death [10],[15],[16]. It is a zoonotic agent whose main reservoirs are cattle and other ruminants (sheep, goats, deer) [3]. The outbreaks recorded until now are related mostly to the bovine and sheep food consumption. However, the ingestion of other food and water, the direct contact with animals and the human transmission through fecal-oral route, are also implicated [4]. Their virulence are linked to the presence of genes coding for intimin (*eae* genes) and toxins (*Stx1*, *Stx2* or *Stx1* and *Stx2* genes) and other virulence genes [9].

In Algeria, cattle seem to be a reservoir of STEC O157 potentially pathogenic. A prevalence of 7.8% was demonstrated on bovine carcasses [5]. Similarly, the search of these pathogens in sheep is of particular interest as this population makes more than 70% of the total workforce with more than 12 million of sheep [12]. A better knowledge of the strains (STEC O157) (identification and antibiotic sensitivity) that circulate in the environment deserves attention in order to better assess health risks in the food chain.

The aim of this study concerns the antibiotic resistance of *Escherichia coli* O157 strains isolated from sheep carcasses for human consumption in two slaughterhouses in Algiers (Algeria).

An initial study showed that the ruminants are an important source of *Escherichia coli* O157 Shiga toxin producers in Algeria [5]. The study of the porting of STEC in stool sheep material showed the presence of *Stx* gene in 27 samples isolated from 106 feces samples tested by multiplex PCR (*eae*, *Stx1*, *Stx2*) with 25, 4% of prevalence.

The second study performed by [7] revealed the presence of *Escherichia coli* O157: H7 in eleven sheep carcasses (n=151) tested in two slaughterhouses in Algiers (Algeria), with 7.26% of prevalence.

Thirteen (n=13) *E. coli* O157 strains carrying at least one virulence genes have been studied to answer to a second problem that is the assessment of antibiotic resistance.

## MATERIALS AND METHODS

The sensitivity of 13 *E. coli* O157: H7 strains isolated from 11 sheep carcasses was tested by using 31 antibiotics belonging to different classes, the diffusion method Disk agar Mueller-Hinton (CM337, Oxoid) using NCCLS standards (National Committee for Clinical Laboratory Standards) recommended by WHO was adopted. Antibiotic discs used are the following: ampicillin (10 µg), amoxicillin / clavulanic acid (20 µg + 10 µg), mecillinam (10 µg), Ticarcillin (75 µg), piperacillin (10 µg), Cefazolin (30 µg), Cephalexin (30 µg), Cefoxitin (30 µg), ceftazidime (30 µg), cefepime (30 µg), cefuroxime (30 µg), cefotaxime (30 µg), aztreonam (30 µg), latamoxef (30 µg), Imipenem (10 µg), gentamicin (10 U), Amikacin (30 µg), Netilmicin (30 µg), kanamycin (30 U) isepamicin (30µg), furans (300µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), Evofloxacin (5 µg), tetracycline (30 U), chloramphenicol (30 µg), sulfonamides (200 µg), Trimethoprim (5 µg), cotrimoxazole (23.75 / 1.25 µg ), colistin (10 µg), fosfomycin (200 µg). Quality control was carried out by the *Escherichia coli* ATCC 25922 strain (sensitive to all antibiotics tested).

After the incubation of the plates at 35C° for 18 hours, the reading was performed using a special device "Osiris", which measures the zones of inhibition of bacterial growth by antibiotics and compared them to the critical values, and therefore classified the bacteria in susceptible, intermediate or resistant.

The MIC was made for all the strains that introduced an antibiotic resistance using the E test® for determining the MIC through the use of impregnated strips of a continuous exponential gradient of the antibiotics tested.

The inhibition of the growth of bacteria results on an inhibition ellipse whose points of intersection with the strip define the CMI. The reading scale printed on the strip enables a rapid interpretation. It should read the value of the MIC that corresponds to the intersection of two ellipses, and then compare it with the critical values of MIC for *Enterobacteriaceae*.

## RESULTS

The results of the antibiogram showed that the strain number 2 is resistant to tetracycline (MIC = 6 g / l), the strain number 4 is resistant to tetracycline (MIC = 6 g / l), the strain number 7 is resistant to furan (MIC = 64 mg / l), the strain 8 is resistant to tetracycline (MIC = 8 g / l) and the strain number 9 is resistant to furans (MIC = 64 mg / l) and tetracycline (MIC = 6 mg / l).

The characterization of *E.coli* O157: H7 strains and their sensitivity to antibiotics are reported in table 1:

**Table 1: characterization of STEC O157 strains**

Strains	Serotype	Sorbitol and $\beta$ -glucuronidase	Pathotype	Antibiotic resistance	Slaughterhouses
1	O157:H7	–	<i>eae stx<sub>2</sub></i>	S	Slaughterhouse n°1
2	O157:H7	–	<i>eae stx<sub>1</sub></i>	R tetracycline	
3	O157:H7	–	<i>eae stx<sub>2</sub></i>	S	Slaughterhouse n°1
4	O157:H7	–	<i>eae</i>	R tetracycline	
5	O157:H7	–	<i>eae stx<sub>2</sub></i>	S	Slaughterhouse n°2 n°2
6	O157:H7	–	<i>eae stx<sub>2</sub></i>	S	Slaughterhouse n°2
7	O157:H7	–	<i>eae stx<sub>2</sub></i>	R furane	Slaughterhouse n°2
8	O157:H7	–	<i>stx<sub>2</sub></i>	R tetracycline	Slaughterhouse n°2
9	O157:H7	–	<i>eae stx<sub>2</sub></i>	R tetracycline R furane	Slaughterhouse n°2
10	O157:H7	–	<i>eae stx<sub>2</sub></i>	S	Slaughterhouse n°2
11	O157:H7	+	<i>stx<sub>2</sub></i>	S	Slaughterhouse n°2
12	O157:H7	+	<i>stx<sub>2</sub></i>	S	Slaughterhouse n°2
13	O157:H7	+	–	S	Slaughterhouse n°2

## DISCUSSION

The Ruminants, cattle and sheep, are a major reservoir of *E. coli* O157 Shiga toxin producers in Algeria. The prevalences found in carcasses 7, 8% for cattle by [5] and 7.2% for sheep [7] are important. A major outbreak of infections due to *E. coli* O157: H7 linked to a contact with sheep, to a consumption of sheep meat or to a by-products, have already been described in all regions of the world [1], [6], [7].

The reservoirs of ruminants carrying O157 STEC are important, Neither the diagnosis in hospitals, nor outbreaks have been described nowadays.

This study confirmed a dual impact that can represent the circulation in the environment of STEC O157 potentially pathogenic strains and also the antibiotic resistant strains in food. From the 13 strains of STEC O157 isolated, 23.1% were found resistant to tetracycline, 7.7% to furan and 7.7% resistant to both of tetracycline and furan. Little work has been done on the resistance antibiotic of *E. coli* O157. Nevertheless, the study in Jordan on multidrug resistance of serotype O157: H7 strains isolated from sheep, revealed that five strains were resistant to ampicillin and streptomycin, one strain to co-trimoxazole, one strain to ampicillin, and one strain to ampicillin-sulbactam cephalosporins (cefazolin, cefuroxime), aztreonam, sulfonamides, cotrimoxazole, aminoglycosides, tetracycline and chloramphenicol [13]. In Iran, 327 samples of feces isolated from ruminants (buffalo, camels, cattle and sheep) were examined in order to look for *E. coli* O157: H7 / NM and to test their sensitivity to the antibiotics. Twenty-five (7.6%) *Escherichia coli* O157: H7 / NH were isolated and 56.0% were resistant to gentamycin, 48.0% to ampicillin, 40.0% to erythromycin, 16.0% to amoxicillin, 12.0% to tetracycline, 8% to chloramphenicol, 8.0% to nalidixic acid, and 4 % to streptomycin. All *E.coli* O157 isolated were susceptible to cefuroxime [14]. The results showed that the resistance of *E. O157* to tetracycline was found in all studies. In fact, tetracycline are used abusively in livestock feed in many countries, resulting in the emergence of resistance among workers (farmers, slaughterers of animals) and consumers of meat and milk. This has led some countries, like the United Kingdom, to prohibit the utilization of this antibiotic in livestock feed [11].

## CONCLUSION

The presence of pathogenic *E.coli* strains resistant to antibiotics poses a double public health problem. In addition to the pathogenicity, the circulation of strains resistant to antibiotics in healthy animals promotes the dissemination of these factors of resistance to other species. Risk analysis to all the links of the production chains and appropriate control plans must be implemented to minimize contamination and spread of these strains. Complementary and comprehensive studies through the development of diagnostic tools should be conducted in both human and veterinary level.

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