

# Hygienic quality of food from animal origin and antibiotic resistance of *Escherichia coli* in a border region of Algeria

## Calidad higiénica de los alimentos de origen animal y resistencia a los antibióticos de *Escherichia coli* en una región fronteriza de Argelia

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### ABSTRACT

Food of animal origin such as milk and meat have a high nutritional value and form an important part of the human and animal diet, but are difficult to produce and are highly perishable. Additionally significant socio-economic loss will result if production and/or storage conditions are neglected, whether through loss of the food or illnesses caused by consumption and treatment. It was in this context that we carried out this study, to assess the hygienic quality of cow's milk and meat produced and consumed in a border region of Algeria. A total of 130 samples were taken from animal foodstuffs (raw cow's milk, sheep carcasses, chicken and turkey meat) at farm, abattoir and butchery levels. Mesophilic aerobic flora, total coliforms, thermotolerant coliforms and *Escherichia coli* were enumerated, and the sensitivity of the *E. coli* to certain antibiotics most commonly used in human and veterinary medicine was assessed. High levels of contamination and bacterial loads ranging from  $5.36 \times 10^2$  CFU·mL<sup>-1</sup> for milk, to  $1.56 \times 10^5$  CFU·cm<sup>-2</sup> for sheep meat, some of this foodstuffs are acceptable but represent a food hazard, and others are not acceptable according to regulations. A high percentage of multiresistant strains and worrying resistance rates were detected, and if the necessary measures are not taken as a matter of urgency in the context of "One Health", the situation is likely to worsen and human and animal health will be affected.

**Key words:** Food of animal origin; microbiological quality; *E. coli*; antibiotic resistance; Algeria

### RESUMEN

Los alimentos de origen animal como la leche y la carne tienen un alto valor nutricional y forman una parte importante de la dieta humana y animal, pero son difíciles de producir y muy perecederos. Además, si se descuidan las condiciones de producción y/o almacenamiento, el resultado será una pérdida socioeconómica significativa, ya sea por la pérdida de alimentos o por enfermedades causadas por el consumo y el tratamiento. Fue en este contexto que llevamos a cabo este estudio, para evaluar la calidad higiénica de la leche y la carne de vaca producidas y consumidas en una región fronteriza de Argelia. Se tomaron un total de 130 muestras de alimentos de origen animal (leche cruda de vaca, canales de oveja, carne de pollo y pavo) a nivel de granja, matadero y carnicería. Se enumeraron la flora aeróbica mesófila, los coliformes totales, los coliformes termotolerantes y *Escherichia coli*, y se evaluó la sensibilidad de las *E. coli* a ciertos antibióticos más comúnmente utilizados en medicina humana y veterinaria. Altos niveles de contaminación y cargas bacterianas que van desde  $5,36 \times 10^2$  CFU·mL<sup>-1</sup> para la leche, hasta  $1,56 \times 10^5$  CF·cm<sup>-2</sup> para la carne de ovino, algunos de nuestros alimentos son aceptables pero representan un peligro alimentario, y otros no son aceptables según las regulaciones. Se detectó un alto porcentaje de cepas multirresistentes y tasas de resistencia preocupantes, y si no se toman urgentemente las medidas necesarias en el contexto de "Una sola salud", es probable que la situación empeore y la salud humana y animal se vea afectada.

**Palabras clave:** Alimentos de origen animal; calidad microbiológica; *E. coli*; resistencia antibiótica; Argelia

## INTRODUCTION

Foodstuffs of animal origin are highly nutrient dense and have excellent taste qualities. However, they are subject to contamination and inevitable bacterial attack as they contain elements necessary for their development [1]. Their presence in a food has three consequences: enhancement, as in the case of fermented foods, or degradation, as in the case of contamination by bacteria with high enzymatic power such as mesophilic aerobic flora, psychrotrophs and *Escherichia coli*, or containing a health hazard without any organoleptic modification of the food, such as *Mycobacterium tuberculosis*, *Brucella* (causative of brucellosis) or certain species of virulent *E. coli*. This is why inspection and microbiological analysis of foodstuffs is so important, from production to consumption, or from the farm to the table [2].

The initial number of bacteria and their species determine the shelf life of a foodstuff. In order to guarantee a healthy, safe, nutritious and sufficient food supply this concept is enshrined as one of the four pillars of food safety [3]. Consumption of food contaminated with undesirable bacteria can lead to various types of food poisoning, ranging in severity from a simple disturbance of the intestinal microbiota in the form of diarrhea to the infection and invasion of the entire organism. It is estimated that one person in ten falls ill after consuming contaminated food, causing 420,000 deaths every year, along with economic losses [4]. In either events mentioned above, antibiotics are used to eliminate the undesirable bacteria which may then be replaced by the normal flora. Concerningly, this approach can fail to work, especially in regions without access to advanced medical care, due to the increasing emergence of antibiotic-resistant strains of bacteria causing foodborne illness.

The United Nations is attempting to remedy this situation by proposing a "One Health" approach, and it is in this context that this study was carried out, to determine the hygienic quality of raw milk and meat of different animal origin consumed in a border region of Algeria, namely Souk Ahras, and to assess the antibiotic resistance of *E. coli* isolated from food of animal origin [5].

## MATERIALS AND METHODS

### Area and period of study

This study took place at the Institute of Agricultural Sciences and Veterinary Sciences, laboratory of Animal Productions, Biotechnologies and Health (PABIOS), Mohamed-Cherif Messaadia University – Souk Ahras, Algeria, from March 2019 to September 2020, and from September 2021 to June 2022 (due to COVID19).

### Sample collection

A total of 130 samples were taken from the Souk Ahras region, including 40 samples of raw cow's milk (*Bos taurus*), 30 samples of sheep (*Ovis aries*) meat, 30 samples of chicken (*Gallus gallus domesticus*) meat and 30 samples of turkey (*Meleagris gallopavo domesticus*) meat.

Samples of raw cow's milk were taken after udder cleaning and disinfection on three farms, and the milk found apparently fit for consumption, i.e. no change in organoleptic characteristics was observed. Ovine carcasses were sampled using a sterile scalpel blade [6] just after stamping, at the Souk Ahras municipal slaughterhouse. Samples of chicken and turkey meat were taken from various butcher's shops in the region, to reflect the same conditions of sale to the

consumer. Each sample was placed in a sterile bottle, marked with an identifier, placed in a cooler with ice packs (Abbott-ICECATCH) and sent to the laboratory.

### Sample processing

All samples were processed in the laboratory within a few hours of collection: Milk samples were diluted directly with peptone water to obtain the various decimal solutions. For sheep meat, a volume of 100 mL of sterilized peptone water was added to each sample to obtain a  $10^{-1}$  stock solution, then different decimal dilutions were prepared. For chicken and turkey meat, the sample in its bottle was weighed (Pionner™, Plus Precision Ohaus®, USA), the weight of the sample is deducted, and a volume (peptone water) of nine times the weight of the sample was added to obtain a  $10^{-1}$  stock solution, in order to avoid any manipulation of the sample and any modification of the existing flora. Decimal solutions were then prepared [7].

### Enumeration of mesophilic aerobic flora, coliforms, thermotolerant coliforms and *E. coli*

One (1) mL of each decimal dilution was inoculated on to PCA (Plate Count Agar) culture medium (Institut Pasteur Algérie) and incubated (UNB 400, Memmert, Germany) at 30°C for 72 hours (h) for mesophilic aerobic flora (MAF) enumeration. All colonies were counted [8].

One (1) mL of each dilution was placed on a Petri dish before pouring a first layer of Violet Red Bile Lactose Agar medium (VRBL) (Difco) and mixing with a second layer added after gelling. Petri dish were then incubated at 30°C for 24 to 48 h for coliforms enumeration and at 44°C for 24 to 48 h for thermotolerant coliforms enumeration. All pink-red colonies with a diameter greater than or equal to 0.5 mm were counted [8].

Enumeration was performed using the following formula  $N = \Sigma C / V \times 1.1 \times d$ , on plates with 10 to 300 colonies [9].

N (CFU): number of microorganisms present in the sample.

ΣC: sum of colonies counted from the two dilutions retained.

V: volume inoculated (1 mL).

d: the dilution rate of the first dilution retained for the counts.

For raw cow's milk, dilutions  $10^{-1}$  and  $10^{-2}$  were retained for the enumeration of MAF, coliforms and thermotolerant coliforms.

For chicken and turkey meat, dilutions  $10^{-2}$  and  $10^{-3}$  were retained for the counts of MAF, coliforms and thermotolerant coliforms.

Dilutions of  $10^{-3}$  and  $10^{-4}$  were used for the enumeration of MAF in ovine meat, and dilutions of  $10^{-2}$  and  $10^{-3}$  were used for the enumeration of coliforms and thermotolerant coliforms.

For *E. coli* identification, colonies of thermotolerant coliforms were isolated and purified for identification on the API E20 gallery (BioMérieux, France), then their counts were deduced.

### Evaluation of antibiotic resistance

Antibiotic resistance of identified *E. coli* strains was assessed on Muller-Hinton agar according to CLSI performance standards [5], after reviving the preserved strains.

The antibiotic discs (Liofilchem, Roeseto, Italy) used were: Penicillin (P)(10); Ampicillin (AMP)(10); Amoxicillin/clavulanic acid (AMC)(20/10); Cefoxitin (FOX)(30); Gentamycin (CN)(10); Kanamycin

(K)(30); Ofloxacin (OFX)(5); Erythromycin (E)(30); Tetracycline (TE) (30); Sulfonamide (SMZ)(50); Sulfonamides/Trimetoprim (SXT)(1.25/23.75); Chloramphenicol (C)(30); Fosfomicin (FOS)(50).

Statistical processing of the results, i.e. comparison of means, analysis of variance and degree of similarity of the *E. coli* strains identified, was carried out using STATISTICA 7 software (Statsoft, France) [10].

## RESULTS AND DISCUSSION

### Prevalence of contamination and bacterial enumeration

The contamination rates of various foods of animal origin (TABLE I) is shown in FIG.1.

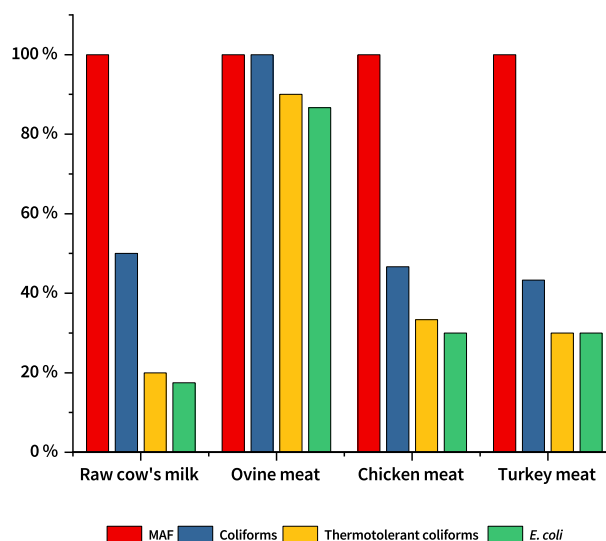


FIGURE 1. Contamination rates of various food of animal origin

In some African countries, extremely high bacterial loads of MAF, coliforms and thermotolerant coliforms in milk have been reported [15, 16, 17] with average loads of *E. coli* reaching  $2 \times 10^6$  CFU·mL<sup>-1</sup> ( $6.3 \text{ Log}_{10}$  CFU·mL<sup>-1</sup>) in raw milk at farm level in Ethiopia [15].

Milk from a healthy animal may contain microorganisms, even if sampled in good conditions. These are saprophytic germs of the udder and galactophore ducts and can reach bacterial loads of  $10^3$  CFU·mL<sup>-1</sup> [8] to  $10^5$  CFU·mL<sup>-1</sup> [18].

It should be remembered that the initial number of bacteria in a food product significantly affects its freshness, add to this the numerous manipulations and the time it takes to reach the processing industries [19], which enhance a bacterial multiplication, and if the product is immediately sold to public markets, a food poisoning is most likely to occur.

All sheep carcasses were contaminated with MAF and coliforms, with average bacterial loads of  $8.34 \times 10^4$  CFU·cm<sup>-2</sup> and  $6.2 \times 10^3$  CFU·cm<sup>-2</sup>, respectively. The rate of contamination by thermotolerant coliforms ( $5.08 \times 10^3$  CFU·cm<sup>-2</sup>) and *E. coli* ( $5.17 \times 10^3$  CFU·cm<sup>-2</sup>) was 90 and 86.66%, respectively.

The counts of MAF, total coliforms, thermotolerant coliforms and *E. coli* identified in the various foods of animal origin are shown in TABLE II with their maximum and minimum values.

In Algeria, similar contamination rates for milk were reported [11, 12] but with higher bacterial loads for MAF and thermotolerant coliforms [11, 13]. The average load of thermotolerant coliforms in this samples is close to that reported by Aggad *et al.* [12] ( $2 \times 10^2$  CFU·mL<sup>-1</sup>), and according to Algerian regulations regarding the microbiological criteria applied to foodstuffs established in the Official Journal of the Republic of Algeria N°39 (Art. 6, Annex 1) these results are satisfactory [14]. Additionally, but the problem resides in the bacterial species known by its opportunistic and pathogenic aspect that colonize the udder and cause mammary gland infection at the slightest opportunity.

TABLE II  
Average loads of MAF, Total coliforms, thermotolerant coliforms and *E. coli* isolated from various foods of animal origin

	MAF (mean ± sd)	Total coliforms (mean ± sd)	Thermotolerant coliforms (mean ± sd)	<i>Escherichia coli</i> (mean ± sd)
Raw cow milk (CFU·mL <sup>-1</sup> )	$5.36 \times 10^2 \pm 2.62 \times 10^2$ * Min: $2.1 \times 10^2$ Max: $1.26 \times 10^3$	$5.16 \cdot 10^2 \pm 2.20 \times 10^2$ * Min: $2.27 \times 10^2$ Max: $1.03 \times 10^3$	$5.69 \times 10^2 \pm 1.57 \times 10^2$ * Min: $3.00 \times 10^2$ Max: $8.64 \times 10^2$	$5.82 \times 10^2 \pm 1.65 \times 10^2$ * Min: $3.00 \times 10^2$ Max: $8.64 \times 10^2$
Ovine carcass (CFU·cm <sup>-2</sup> )	$8.34 \times 10^4 \pm 3.48 \times 10^4$ **** Min: $2.9 \times 10^4$ Max: $1.56 \times 10^5$	$6.2 \times 10^3 \pm 2.65 \times 10^3$ **** Min: $2.64 \cdot 10^3$ Max: $1.11 \times 10^4$	$5.08 \times 10^3 \pm 1.98 \times 10^3$ *** Min: $2.36 \times 10^3$ Max: $9.36 \times 10^3$	$5.17 \times 10^3 \pm 1.95 \times 10^3$ * Min: $2.36 \times 10^3$ Max: $9.36 \times 10^3$
Chiken meat (CFU·g <sup>-1</sup> )	$8.6 \times 10^3 \pm 3.52 \times 10^3$ *** Min: $3.45 \times 10^3$ Max: $1.65 \times 10^4$	$8.64 \times 10^3 \pm 1.73 \times 10^3$ *** Min: $5.09 \times 10^3$ Max: $1.09 \times 10^4$	$6.5 \times 10^3 \pm 2.04 \times 10^3$ *** Min: $3.09 \times 10^3$ Max: $9.82 \times 10^3$	$6.13 \times 10^3 \pm 1.77 \times 10^3$ *** Min: $3.09 \times 10^3$ Max: $8.45 \times 10^3$
Turkey meat (CFU·g <sup>-1</sup> )	$5.25 \times 10^3 \pm 2.7 \times 10^3$ ** Min: $3.18 \times 10^3$ Max: $1.35 \times 10^4$	$3.53 \times 10^3 \pm 6.36 \times 10^2$ ** Min: $2.64 \times 10^3$ Max: $4.73 \times 10^3$	$3.47 \times 10^3 \pm 6.47 \times 10^2$ ** Min: $2.55 \times 10^3$ Max: $4.45 \times 10^3$	$3.47 \times 10^3 \pm 6.47 \times 10^2$ ** Min: $2.55 \times 10^3$ Max: $4.45 \times 10^3$

sd: standard deviation. \*: significant difference ( $P < 0.05$ ) read vertically, except for chicken and turkey meat, read vertically and horizontally (CFU·g<sup>-1</sup>, both)

This results are close to those reported by Harhoura *et al.* [20] in Algeria, and Jaja *et al.* (2018)[21] in South Africa, but lower bacterial loads were reported by Djenidi (2016)[22], Nouichi *et al.* (2009)[23] and El Hadeif *et al.* [24], in Algeria.

Contamination of carcasses by MAF is inevitable, but contamination by coliforms, thermotolerant coliforms and *E. coli* means contamination by intestinal contents, which are the result of a lack of hygiene during slaughtering, skinning, and evisceration. At the slaughterhouse, we noticed the absence of specific clothing for personnel, a single knife and a sharpening rifle tool for all stages of primary processing, failure to perform the forward motion principle, with sheep being worked on the floor in dorsal recumbency, no clear separation between the clean and soiled sectors, and a single slaughter room where all operations are carried out, including the stamping and weighing of ovine carcasses.

For the tested white meats, there was no significant difference between the contamination rate of chicken and turkey meat, but the bacterial load of chicken meat was higher than that of turkey meat ( $P<0.05$ ).

Contamination rates higher than these results were reported in Algeria [25, 26], Morocco [27] and Mexico [28]. This results are similar to those reported in Italy [29], Sri Lanka [30], Thailand [31], and Bangladesh [32] for frozen chicken meats, bearing in mind that cold does not sanitize the food, hence the importance of refrigerating and freezing a healthy food as soon as possible and without interruption of cold throughout the storage period.

Higher MAF and *E. coli* bacterial loads were reported in western Algeria [33], but lower bacterial loads are reported in eastern Algeria [26]. This results are close to those reported in Turkey by Eyi and Aslan [34], and in view of Algerian [14] and European [35] regulations, these chicken and turkey meats are not acceptable given the high bacterial load, to be specified that in Algerian regulations, only the bacterial load of *E. coli* is mentioned [14].

The bacterial loads reported in this study indicate poor hygiene and/or handling conditions, and it is difficult to identify where hygiene

conditions have failed, because the traceability and origin of these meats is unknown, even if butchers have a health certificate, but the absence of packaging and labels make identification impossible, especially that many slaughtering is carried out clandestinely in order to increase the profit margin.

### Antibiotic resistance

The resistance frequencies of the *E. coli* identified towards the antibiotics tested are shown in the TABLE III and the resistance profiles of the *E. coli* strains towards the antibiotics are shown in the dendrogram in the FIG. 2 while studying the degree of approximation of the *E. coli* strains identified.

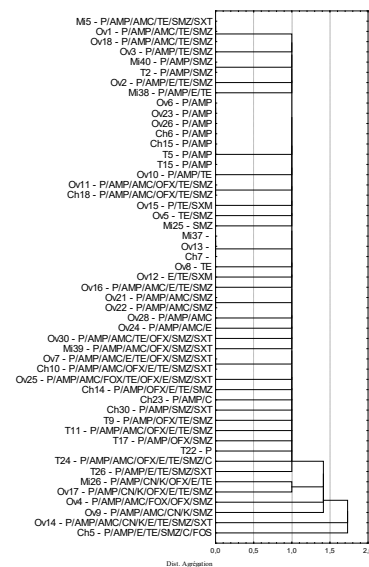


FIGURE 2. Degree of similarity between *Escherichia coli* strains identified according to their resistance profiles to the antibiotics tested

TABLE III  
Resistance frequencies of the *Escherichia coli* identified from different animal origin foodstuff

Antibiotic family	Antibiotics	Milk (07 strains)	Ovin meat (26 strains)	Chicken meat (09 strains)	Turkey meat (09 strains)	Total (51 strains)
Beta-lactam	Penicilline <sup>b</sup>	71.42	84.61	88.88	100	86.27
	Ampicilline <sup>b</sup>	71.42	80.76	88.88	88.88	82.35
	Amoxycilline/ Clavulanic acid <sup>d</sup>	28.57	53.84	22.22	22.22	39.21
	Cefoxitin <sup>a,b</sup>	0	7.69	0	0	3.92
Aminoglycosides	Gentamycin <sup>b</sup>	14.28	11.53	0	0	7.84
	Kanamycin <sup>b</sup>	14.28	11.53	0	0	7.84
Fluoroquinolone	Ofloxacin <sup>d</sup>	28.57	23.07	33.33	44.44	29.41
Macrolides	Erythromycin <sup>d</sup>	28.57	30.76	33.33	33.33	31.37
Tetracyclin	Tetracyclin <sup>f</sup>	57.14	61.53	44.44	44.44	54.90
Sulfonamides	Sulfamide <sup>g</sup>	57.14	69.23	55.55	66.66	64.70
	Sulfamide/triméthoprim <sup>e</sup>	28.57	15.38	22.22	11.11	17.64
Phenolics	Chloramphénicol <sup>b,b</sup>	0	0	22.22	11.11	5.88
Fosfomycine	Fosfomycin <sup>a</sup>	0	0	11.11	0	1.96

a,b,c,d,e,f,g,h: significant difference ( $P<0.05$ )



Resistance assessment of isolated *E. coli* strains showed that 72.55% of strains were multiresistant as defined by CLSI [36], 15.67% were double-resistant, 5.88% were resistant to a single antibiotic and 5.88% were susceptible to all antibiotics assessed. Multi-resistant strains present a medical hazard, because in the event of human or animal infection, it will be difficult to obtain satisfactory results without the prior use of antibiotic susceptibility testing, especially as these strains can cause food poisoning and/or dangerous foodborne infections. This multiple antibiotic resistance is mainly due to unregulated use in humans, animals, and agriculture [37, 38].

The *E. coli* strains isolated showed high levels of resistance to penicillin (86.27%), ampicillin (82.35%) and, to a lesser extent, sulfonamides (64.70%). This resistance is due to their availability on the market and their frequent use on farms at the slightest sign of possible disease, especially by breeders. Some of the authors have also reported high rates of resistance for these antibiotics, ranging from 70 to 100% resistance for *E. coli* isolated from milk and meat, notably in Algeria [39, 40, 41], Mexico [28] and Brazil [42]. Lower resistance rates are reported in Bangladesh [32].

A resistance of 39.21% was observed for amoxicillin associated with clavulanic acid, in Algeria, lower prevalences of resistance are reported [25, 39, 41] for *E. coli* strains isolated from milk and meat, but also, high resistance prevalences are reported by Dib *et al.* [40], as well as Abimbola *et al.* 2023 (98.1%) [43] in Nigeria, Martínez-Vázquez *et al.* [28] (100%) in Mexico and Hussein *et al.* (2023) [44] (76%) in Lebanon, having worked on cheese.

This results are close to those reported by Worku *et al.* [45] in Ethiopia, and by Obaidat *et al.* [46] in Jordan in imported beef. In Algeria, this antibiotic is being used exclusively in humans, especially children, and is included in the list of medicines subject to compulsory medical prescription, for this reason it cannot be ruled out that this contamination is of human origin, either directly during processing or indirectly at farm level.

For Ofloxacin, resistances far exceeding these results (29.41%) are reported in Algeria [39, 41], Uganda [47] and Sri Lanka [30] for strains isolated from various white and red meats, and milk. This results are close to those reported in Morocco [27] in chicken products, Bangladesh [32] in frozen chicken and Jordan [46] in imported beef. Lower levels of resistance have been reported in Algeria [25], Mexico [28] and Lebanon [44] for strains isolated from cheese. These resistances are due to the use of quinolones in poultry farming [30] and significantly in Africa [37].

Erythromycin resistance is 31.37%, resistance rates of 60, 98.72 and 100% reported in Bangladesh [48], Algeria [40] and Saudi Arabia [49], respectively, in contrast to those reported in Morocco with a resistance rate of 1% [27] for *E. coli* strains isolated from chicken products. Erythromycin is the antibiotic of choice used by veterinarians for respiratory diseases in Algeria, and failure to comply with rearing conditions leads to repeated respiratory ailments and repeated use of this antibiotic, which will result in prominent levels of resistance throughout the country in the years to come.

For oxytetracycline, 54.90% resistance is reported, this results are close to those reported in Algeria by Boudjerda and Lahouel [25] (64.25%), in Ethiopia by Asfaw *et al.* [50] (52.5%), in Bangladesh by Hossain *et al.* [32] (66%) and in Mexico by Martínez-Vázquez *et al.* [28] (60%). However, lower resistance rates than these results were reported in Algeria [40, 41] and Morocco for chicken meat products

[27]. Aberkan *et al.* [39] and Boudjerda and Lahouel (2015) [51] reported resistance of 100 and 96.41% in Algeria, respectively, as did Worku *et al.* (2022) [45] (100%) in Ethiopia. These resistances are due to overuse of this antibiotic, taking into account the fact that in 2016, oxytetracycline accounted for 63% of the antibiotic used in Africa [37].

An exceptionally low resistance rates were found for Cefoxitin (3.92%), Gentamycin (7.84%), Kanamycin (7.84%), Sulfonamides associated with Trimethoprim (17.64%), Chloramphenicol (5.88%) and Fosfomycin (1.96%), these results are close to those reported in Algeria [39, 41]. This low level of resistance can be explained by the fact that these antibiotics are unfamiliar to breeders, less used by veterinarians and doctors, unavailable in pharmacies and only for hospital use in injectable form, or completely banned like chloramphenicol.

However, some Algerian authors who have isolated *E. coli* from chicken meat have reported high resistance rates for some of these antibiotics [40, 51], which are worrying, especially with the irrational and clandestine use of certain banned antibiotics in Algeria.

## CONCLUSIONS

Food safety, food processing and antibiotics, all of it, represent risks for public health, and concern all human beings, which arise the importance of adopting "One Health" concept and the necessity of implementing national and international programs, conventions, and aids in developing countries.

Our results show a low level of hygiene in meat produced and marketed in the Souk Ahras region, and indicate a high risk for consumers.

This type of meat also represents a potential reservoir for multidrug resistant *E. coli*, which can be transferred to humans and cause poisoning and/or infections.

The application of good production and hygiene practices throughout the food chain is becoming an absolute necessity, as is the training and awareness-raising of professionals in the sector, and the provision of information to consumers. This will help reduce the number of illnesses caused by these meats, and curb the spread of multi-resistant strains, which are becoming increasingly worrying.

## Conflicts of interest

The authors declare no conflict of interest.

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