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Prevalence of *Escherichia coli* Enterohemorragic O157:H7 in Frozen Bovine Meat in Algeria

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Abstract: The presence of Escherichia coli O157: H7 in frozen imported meat was studied in north-western Algeria. Two hundred and fifty one samples were tested for the presence of E. coli serogroup O157: H7 using the standard method of culture and serotyping technique. The pathogen was detected in 0.44% of samples. The isolate strain was nonsorbitol fermenter, O157 and H7 agglutinating. It was tested using the method of KIRBY and BAUER according to the nccls standards to view resistance to antibiotics and was considered sensitive to the ten antimicrobial agents (ampicillin, chloramphenicol, gentamycin, colistin, flumequine, enrofloxacin, nitrofurantoin, cephalothin, trimethoprim-sulfamethoxazole and tetracycline). This study confirms that frozen imported meat is an important reservoir of E. coli O157:H7 pathogenic for human.

Key words: *Escherichia coli* O157:H7 · Identification · Serotype · Frozen imported meat · Algeria

INTRODUCTION

The first *E. coli* enterohaemorrhagic (EHEC) occurred in 1982 in the United States (Oregon and Michigan), with two outbreaks of severe hemorrhagic colitis, after consommation undercooked hamburgers from a fast food products. A strain of Escherichia coli of a new serotype O 157: H7 was detected in the faeces of patients and in beef burgers [1]. Other study on the of children faeces suffering from haemolytic uremic syndrome (HUS), showed the presence of *E. coli* that producing a cytotoxic toxin to Vero cell cultures, hence the name "verotoxin" also known as Shiga-toxin because of its close similarity to a toxin produced by *Shigella dysenteriae* [2]. Enterohaemorrhagic *Escherichia coli* O157: H7 is a human pathogen that causes no apparent disease in cattle, its primary reservoir host.

The sources of *E. coli* O157 :H7 are mainly the products of animal origin such as hamburgers from an undercooked beef [3-8], dairy products (cheese from raw milk) [9,10], OVO-products but also products of plant origin such as cider apples, lettuce, potatoes, radish shoot [11-15].

On the other hand, the external environment is an important source, especially through water swimming or wading in lakes contaminated with feces of human origin, or inadequately chlorinated drinking water [16-20].

Ruminants, especially cattle are considered at present as the main reservoir of *E. coli* O157:H7 [21].

Given the importance of imported bovine frozen meat in Algeria and the fact that many meat products from this specie was not treated with any previous bacteriological sterilization procedures. The aim of the present study was to investigate the prevalence of STEC O157:H7 in bovine meat in order to determinate the potential risk that the consumption of these products can have from the standpoint of public health and hygiene.

MATERIALS AND METHODS

Sample Collection: Two hundred and fifty one samples of imported frozen meat from Argentina, Brazil, Uruguay, Ireland, New Zealand and Australia were analyzed. The imported meat is placed in containers and placed in refrigerated vehicles during transportation at sea.

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Samples are taken randomly from boxes outside of our region and conveyed respecting the freezing temperature of -18°C to the Regional Veterinary Laboratory of Tlemcen.

During thawing of frozen meat at room temperature, the microbiological samples were performed and tested.

Culture and isolation of *Escherichia coli* O157:H7: Enrichment cultures for each sample were carried out by combining 25 g of each sample with 225 ml of buffered peptone water supplemented into a stomacher bag, homogenized for 2 min and incubated at 37°C for 16–18 h. After incubation, the 251 isolates belonging to *E. coli* were cultured on Sorbitol Mac Conkey medium for detecting non sorbitol variants. This test required the identification guides to the pathogenic EHEC strains. The strains metabolizing sorbitol are excluded, however the rest of the strains are tested for their immunological confirmation by the method of Farmer and Davis [22].

Serological Identification: All biochemically identified non sorbitol fermenting colonies from the SMAC Agar were subjected to slide agglutination with the *E. coli* O157 BD *E. coli* antisera (Difco) and the agglutinating colonies were further processed for definitive confirmation [22].

Antibiotic Susceptibility Test: The isolated strains were tested for ten antibiotics resistance (ampicillin, chloramphenicol, gentamycin, colistin, flumequin, enrofloxacin, nitrofurantoin, cephalotin, sulfamethoxazole-trimethoprim and tetracycline) using the standard Kirby–Bauer method [23].

RESULTS

From 251 samples of meat analysed; 288 gram negative bacterial strains were isolated in which 259 *Escherichia coli* sorbitol positive were characterized by classical phenotypic tests in all samples. Whereas, only one isolated of *Escherichia coli* sorbitol negative was identified and isolated from Uruguay sample (Table 1). This isolate was rod and gram negative nonsorbitol fermenting and produce indole. The agglutinating test showed that this strain is belonging to the serotype of *Escherichia coli* O157 and H7 (Tab: 2). These characters' were specific for the Shiga toxin-producing *Escherichia coli* (STEC), also called verotoxin-producing *E. coli,* is the most important recently emerged group of food-borne pathogens, especially the serotype O157:H7 [24].

The isolated strains were susceptible to the ten antimicrobial agents tested (Table 2): ampicillin, chloramphenicol, gentamycin, colistin, flumequin, enrofloxacin, nitrofurantoin, cephalotin, sulfamethoxazole-trimethoprim and tetracycline.

Table 1: Isolation of *Escherichia coli* 0157:H7 from different sources of imported frozen meat in Algeria

Country	Uruguay	Brazil	Argentina	Ireland	Australia	New Zealand	Total
Number of Samples examined	115	86	36	08	04	02	251
Number of positive samples (%)	1 (0.86)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1

Table 2: Characteristics of *Escherichia coli* and *Escherichia. coli* O157: H7 strains isolated from different sources of imported frozen meat in Algeria

	Reactions	Reactions from other
Antibiotics	Number of strains resistant	Percentage of resistance
Ampicillin	116	44.78
Gentamycin	98	37.83
Chloramphenicol	32	12.35
Colistin	12	4.63
Flumequine	75	28.95
Enrofloxacin	63	24.32
Nitrofurantoin	167	64.47
Cefalexin	20	7.72
Trimethoprim Sulfamethoxazol	151	58.30
Tetracycline	185	71.42
	E. coli O157: H7	*Escherichia coli*
Colonies	Colorless	Pink
Gram stain	Negative	Negative
Methyl red	Red (+)	Red (+)
Voges-Proskauer	Colorless (-)	Colorless (-)
Citrate	Green or no growth (-)	Green or no growth (-)
Sorbitol	Colorless (-)	Colour (+)
O157	Positive (+)	Negative (-)
H7	Positive (+)	Negative (-)

DISCUSSION

Enterohemorrhagic *Escherichia coli* O157:H7 is an important human pathogen that has been isolated from contaminated water as well as meat products. It produces hemorrhagic colitis after oral ingestion and Shiga toxin-producing strains may additionally cause acute renal failure or neurological disturbances especially in young, elderly or immunocompromised individuals [25].

A large number of studies on the incidence and the isolation of *E. coli* O157: H7 from different foods in different countries, Dontorou *et al.* [26] showed that (1.0%) samples of ewes' milk, 1.3% of fresh sausages and 2.0% swine intestines prepared for kokoretsi were contaminated by *E coli* O157 H7.. Abdul-Raouf *et al.* [27] reported that 6% of raw cows' milk samples examined in Egypt were contaminated with *E. coli* O157:H7.

In 2004 Cagneya and collaborators noted the presence of *E. coli* O157: H7 in 2.8% over 1533 samples of beef burger. In Netherlands, 1.1% of 571 samples of raw minced beef were contaminated [28] and in Switzerland, no *E. coli* O157:H7 was isolated from 211 minced beef samples tested [29].

In our study the interest was based on the search of *E. coli* O157: H7 in frozen imported meat considering that consumption of red meat and especially beef' has risen steadily and the stock continues decreasing in Algeria, in order to know if the cold chain was observed during the transport of foods. Apart from the study by Chahed *et al.* [30], on the contamination assessment levels in bovine carcasses with this bacteria in Algeria, where the author has detected two strains of serotype O157: H7 and the others were serotype O157: H-. a total of 230 samples [30]. There is very few data on this subject in North Africa and particularly in Algeria.

On the other hand, from the 251 samples studied, 259 *E. coli* were isolated and only one strain of serotype *E. coli* O157: H7 detected from beef from Uruguay with a prevalence of 0.44%. The presence of a single strain of *E. coli* O157 H7 is due to the satisfactory hygienic quality of the conservation treatment before freezing. And in addition the use of cold act in two ways, one as an agent for growth inhibiting bacteria in which the temperature varies between -10°C and -18°C, in this case the water is transformed into ice, the other is an ultra rapid lowering of the temperature until it reaches -18°C which significantly reduces the rate of bacterial growth.

Regarding the study of the sensitivity of all the isolated bacteria to antibiotics, there is a high percentage of resistance for *E. coli* non O157 especially tetracycline, nitrofurantoin and trimethoprim-sulfamethoxazole with respective rates of 71.42%, 64.47%, 58.30%. And only one resistance was observed for *E. coli* O157: H7 for tetracycline and sensitive to the other antibiotics. However, some studies showed a certain resistance to streptomycin, sulfisoxazole and tetracycline [31, 32]. Other studies have shown a higher rate of antimicrobial resistance in *E. coli* O157 bovine strains compared to human origin strains [32, 33]. Also, they reported that all food resistant strains were isolated from ground beef. Radu et *al.* [34] tested for susceptibility 28 isolates from tenderloin beef and chicken burgers to 14 antibiotics and found that all were resistant to two or more of the tested antibiotics.

Our data on the prevalence of *E. coli* O157:H7 are relatively low on account of good meat processing and the hygiene conditions. Presence in meat of *E. coli* O157:H7, which is involved in human foodborne illness, is considered important. In view of this study monitoring continuum is recommended with the development of specific techniques and the development of a culture medium that enables a rapid and reliable detection of the presence of *E. coli* O157:H7 at even minimal doses.

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