academicJournals

Vol. 10(1), pp. 15-22, 7 January, 2016
DOI: 10.5897/AJMR2015.7839
Article Number: 481C5F656762
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Safety characterization of *Yersinia enterocolitica* strains isolated from raw milk in Western Algeria

Kh. Z. Hadef, Kh. Medjahdi, H. Beldjilali, I. Benamar and B. Moussa-Boudjemâa*

Laboratoire de Microbiologie Appliquée à l'Agroalimentaire, au Biomédical et à l'Environnement (LAMAABE), Université de Tlemcen, 13000 Tlemcen, Algérie.

Received November 2, 2015 Accepted December 16, 2015

***Yersinia enterocolitica*, an important food-borne enteric pathogen is associated with various clinical manifestations ranging from self-limited gastroenteritis to more invasive syndrome such as terminal ileitis and mesenteric lymphadenitis. The main aim of this study was to investigate the incidence of *Y. enterocolitica* in milk samples. For this purpose, one hundred (100) samples of raw cow's milk were collected from the western Algeria region. Seventeen (17) isolates were obtained. All these isolates belong to *Y. enterocolitica* biotype 1A and were *ystB* positive. Heat resistance and antibiotic susceptibility of these isolates were also investigated. The heat resistance D-value (decimal reduction time) and heat sensitivity Z_T values (increase in temperature leads to a ten-fold reduction of the D value) of *Y. enterocolitica* in BHI broth showed that D_{55}, D_{60} and D_{65} were 1.34, 0.85 and 0.62 min, respectively. The obtained Z value was 29.98°C and antibiotic resistance profiles of 17 isolates were evaluated. All the isolates were susceptible to 13 of the 30 tested antibiotic, resistance was noted for eight different antibiotics, among are them Ampicillin and 3rd generation Cephalosporins. The presence of chromosomal *ystB* gene virulence and antibiotic susceptibility indicate that these isolates from raw milk are potentially able to cause human foodborne illnesses and highlights the role of milk as a transmission vehicle of potentially pathogenic *Y. enterocolitica* strains, with consequent risks for consumer's health via the consumption of raw milk and derivatives.**

Key words: *Yersinia enterocolitica,* biotype, virulence gene, heat resistance, antibiotic resistance, raw milk.

INTRODUCTION

Yersinia enterocolitica, which was first described in 1934 as a small Gram-negative coccobacillus psychro-tolerant enterobacterium, isolated from several environmental sources, that is, foods and human clinical samples are a causative organism in several out-breaks of gastroenteritis, in which foods were implicated (Bottone, 1999; Soltan-Dallal et al., 2004; Lambertz and Danielsson-Tham, 2005). In recent years, *Y. enterocolitica* has been the third most common cause of food borne diseases after *Campylobacter* spp.

*Corresponding author. E-mail: b.moussaboudjemaa@gmail.com. Tel: +213 551 626 216.

and *Salmonella* spp. (EFSA, 2011).

Y. enterocolitica has six biotypes, biotypes 1B, 2, 3, 4, and 5 which are known to be pathogenic and those of biotype 1A are considered as nonpathogenic (Bottone, 1999; Soltan-Dallal et al., 2004). The biotype 1A strains are generally regarded as non-virulent. They lack pYV plasmid and major chromosomal virulence genes. Despite this, some biotype 1A strains produce disease symptoms indistinguishable from that produced by known pathogenic biotypes (1B, 2-5). Some biotype 1A strains are able to invade epithelial cells, resist macrophages and carry genes associated with virulence (Tennant et al., 2003; Bhagat and Virdi, 2010). The *ystB* gene is widely distributed in *Y. enterocolitica* biotype 1A strains where the production of *Yersinia* stable toxin Yst-b is the major contributor to diarrhea produced by biotype 1A strains (Singh and Virdi, 2004b).

The frequent association of *Y. enterocolitica* with raw milk (Bernardino-Varo et al., 2013) and the ability of this organism to grow in milk at refrigeration temperatures (Bari et al., 2011) have been well documented. Some *Y. enterocolitica* biotypes are considered as the major prevalent milk-borne pathogens (Bernardino-Varo et al., 2013); they are responsible for gastroenteritis and other syndromes in humans and animals (Huovinen et al., 2010; Singh and Virdi, 2004b). Thus, its control is important for the safety of refrigerated dairy products (Ye et al., 2014). *Y. enterocolitica* has been isolated from raw milk and pasteurized dairy products in several countries e.g. in the USA (Jayarao and Henning, 2001), China (Wang et al., 2010; Ye et al., 2014), Mexico (Bernardino-Varo et al., 2013), Brazil (Falcão et al., 2006), Iran (Soltan-Dallah et al., 2004; Rahimi et al., 2014; Jamali et al., 2015), India (Subha et al., 2009), Turkey (Guven et al., 2010), Nigeria (Okeke and Okwori, 2014), Egypt (Darwish et al., 2015) and other countries. In Algeria, the raw milk is still frequently consumed. National production of raw cow's milk is estimated at 2.3 billion liters. Only a third of this quantity is integrated to the industrial plants (ITELV, 2012), therefore, the most important issue about *Y. enterocolitica* is its control in raw milk and derivatives.

A broad spectrum of antibiotics has been widely used in agriculture to treat infections and improve growth and feed efficiency in livestock and poultry (Mathew et al., 2007). The need to use antibiotics in the treatment of humans and animals may lead to the development of mechanisms resistance antibiotic, causing a growing risk to human and animal health (Perkowska et al., 2011). For this reason, the use of antibiotic growth promoters in animal production must be prohibited or controlled in each country (Singh and Virdi, 2004a). Moreover, *Y. enterocolitica* produces beta-lactamase (penicillinase and cephalosporinase) that make them naturally resistant strains to Penicillins and Cephalosporins first and second generations (Singh and Virdi, 2004a). Systematic monitoring of the susceptibility of bacterial strains, including *Y. enterocolitica*, must therefore be regarded as highly justified to ensure appropriate treatment of humans and to limit the spread of microorganisms' drug resistance in animals (Perkowska et al., 2011).

MATERIALS AND METHODS

Sampling

One hundred (100) raw cow's milk samples were collected aseptically from cans and tanks at the level of the dock receipt of dairy plant "Giplait Mansourah" located in Tlemcen city (northwest of Algeria) Samples were taken in a 250 mL sterile container, then transported to the laboratory in ice boxes. The journey took 15 min. At the laboratory, samples were immediately processed.

Raw cow's milk collected at the dairy is from different areas of Western Algeria: Ain- Temouchent, Ain-Youcef Amieur, Beni Mester, Mansourah, Ouled Mimoun, Remchi, Sabra, Sebdou, Sidi Bel-Abbès and Tlemcen. Milk was brought by farmers in the early morning in cans and refrigerated tank trucks. The study covers the period from January to October 2013. On average, 2 to 3 samples per week were used. The number of farmers is about 700 with an average of 8 cows/ breeder.

Isolation and isolates identification

Y. enterocolitica strains were isolated using two enrichment steps, a pre-enrichment in Peptone Sorbitol Bile salts broth (PSB broth, Fluka, India) and an enrichment in Irgasan, Ticarcillin and potassium Chlorate broth (ITC broth, Fluka, India). One milliliter of each sample was added to 10 mL of PSB broth. The presumptive presence of *Y. enterocolitica* was checked after 4 weeks (28 days) of incubation at 4°C without shaking. One milliliter of each pre-enriched culture in PSB broth was added to 10 mL of ITC broth and incubated at 25°C for 48 h without shaking. In order to reduce the background contaminating flora, Aulisio's alkali treatment method was performed: 0.5 mL of each enriched ITC broth was treated with 4.5 mL of 0.5% KOH solution (prepared in 0.5% NaCl solution), stirred for 20 s (AFNOR, 2003). Then, a loopful of the mixture was streaked immediately on Mac Conkey agar (Fluka, India) and Cefsulodin, Irgasan Novobiocin agar (CIN agar, Fluka, India) and incubated for 24 to 48 h at 30°C (Figure 1). The presumptive isolates were exanimate by biochemical tests as described by the ISO 10273:2003 horizontal method for the detection of presumptive pathogenic *Y. enterocolitica* with the following tests: Gram staining, oxidase, catalase, indole production, tryptophane deaminase, glucose and lactose fermentation, gas formation from glucose, H_2S production, lysine decarboxylase, utilization of Simmons citrate, esculin hydrolysis, reduction of nitrate, mobility at 25 and 37°C and fermentation of xylose, mannitol and trehalose (Table 1) (AFNOR, 2003). The isolates were further identified by using the API 20E (BioMerieux, France). This system is still accepted as the good standard for the rapid identification of *Y. enterocolitica* (Tudor et al., 2008). The identification of biotype relies on a panel of biochemical tests as described in the ISO 10273-2003 method, allowing differentiation of pathogenic biotypes from the non-pathogenic biotype (AFNOR, 2003). The protocol of *Y. enterocolitica* isolation from raw milk and following identification and pathogenicity determination of the isolated strains is schematized in Figure 1.

Real-time PCR for detection of ystB gene

The PCR assays have been developed as an efficient tool for

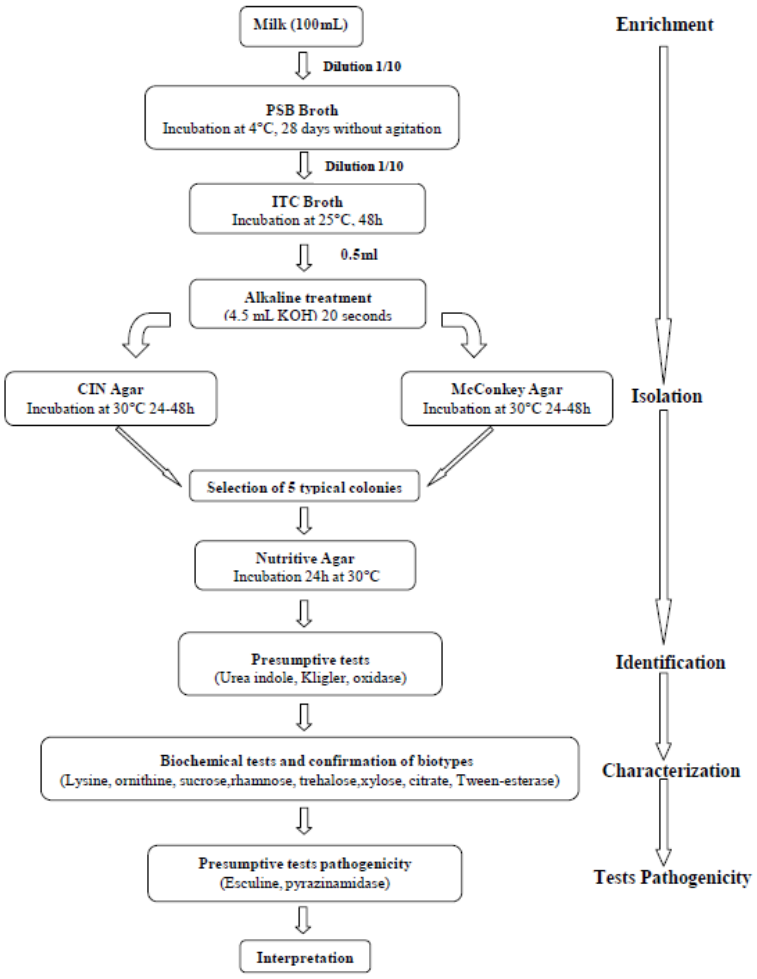

Figure 1. Schematic protocol of *Y. enterocolitica* isolation from raw milk and following identification and pathogenicity determination of the isolated strains.

Table 1. Some phenotypic and biochemical characteristics of *Y. enterocolitica* strains isolated from Algerian raw milk.

Test	Reaction	Test	Reaction
Gram staining	-	Oxidase	-
Utilization of Simmon's citrate	-	Catalase	+
Motility at 37°C	-	Gas production	-
Motility at 25°C	+	ONPG	+
Voges-Proskauer at 37°C	-	Lactose	-
Voges-Proskauer at 25°C	+	Sucrose	+
Lysine decarboxylase	-	Maltose	+
Ornithin decarboxylase	+	Mannitol	+
Urease activity	+	Rhamnose	-
Indole production	+	Arabinose	+
Nitrate reductase	+	Raffinose	-
H_2S production	-	Xylose	+
Kligler test	+	Salicin	-

(+): Positive reaction; (-): negative reaction.

identifying pathogenic *Y. enterocolitica* (Lambertz and Danielsson-Tham, 2005). Real time PCR targeted the chromosomally-located *ystB* gene that is present in all *Y. enterocolitica* strains (Wang et al., 2010). Strains were sub-cultured on (plate count agar) PCA at 30°C for 24 h. DNA was extracted from colonies with QIAamp DNA mini kit (Qiagen, USA) following the manufacturer's instructions. All PCR were performed on a CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, California), in a final volume of 25 µl with the Sybr® Green JumpstartTMTaqReadyMix TM (Sigma-Aldrich, Saint Louis, Missouri). The primers used in this study, were (5′-GTA CAT TAG GCC AAG AGA CG-3′) and (5′-GCA ACA TAC CTC ACA ACA CC-3′) (Baghat and Virdi, 2009). The final concentration of primers in the PCR reaction was 0.2 µM for *ystB*.

The PCR reaction was carried out under the following conditions: an initial denaturation at 95°C for 5 min, 34 cycles of 95°C for 10 s (denaturation), 55°C for 15 s (annealing) and 70°C for 1 min (extension) (Baghat and Virdi, 2009). The PCR products (146 bp) were visualized by ethidium bromide staining on 1.8% TBE agarose gel. Mass values are for 1 µg/lane. A 50- bp DNA ladder (Biolabs, New England) was used to determine the size of PCR product.

Heat treatment

One strain isolated from raw milk (baptized YHK261) was chosen for further characterization because of its unusual heat resistance. This strain was aseptically transferred to 100 mL Brain Heart Infusion Broth (BHI Conda, Spain) and incubated at 30°C for 24 h. 1 mL was sub-cultured in 100 mL BHI at 30°C for 18 h. At the stationary phase, cells were recovered and the culture was adjusted to a final colony count of 10^8 CFU mL^{-1}. Heating temperatures of 55, 60 and 65°C were chosen based on previous studies reported in literature (Pagán et al., 1999). The vials containing 100 mL of sterile BHI were placed in water-bath heated at the preselected temperature (e.g. 55°C). The vials are fixed so that the broth is totally submerged in the bath. The sample temperature during treatment was monitored using a thermometer (IsoLab GmbH, German) placed in another vial containing 100 mL of BHI simultaneously placed in the water bath with the first one, and to minimize any risk of contamination. In a first step, a bacterial suspension was introduced into the heating medium. Samples were removed periodically and immediately placed in ice-water. Each cooled sample was serially diluted in 9 mL of sterile physiological saline (0.9% NaCl w/v). During the third step, dilutions cascade was performed. Direct counts were obtained by plating in duplicate from the dilution series onto trypticase soy agar (TSA Conda, Spain). After incubation at 30°C for 24 h, plates were examined for typical colonies of *Y. enterocolitica*. The number of colony forming units (CFU) on agar plates was converted to $\log_{10}$ CFU g^{-1}. Each experiment was carried out in duplicate at each temperature.

The slope was obtained for each plot of $\log_{10}$ of surviving cells mL^{-1} against time using linear regression analysis Log_{10} D (T). The estimate of thermal resistance was obtained by fitting the linear regressions of the $\log_{10}$ number of surviving cells at each time interval. D values are the absolute value of the inverse slope of the regression line. These D values, in minutes, were used to fit plots of $\log_{10}$ D value versus temperature. To fit the models to the experimental data, the GraphPad PRISM (GraphPad Software, San Diego, CA, USA) was used. D values for *Y. enterocolitica* were calculated using the average slope for a given treatment. The value of the inverse slope obtained by plotting $\log_{10}$ D value versus temperature represents the Z value.

Antibiotic susceptibility testing

Y. enterocolitica isolates were examined for their susceptibility to b-lactam and non-b-lactam antibiotics. Antimicrobial susceptibility was determined by the standard disk diffusion method of Bauer, using Mueller-Hinton agar (Singh and Virdi, 2004a) and antibiotic disks were purchased from Pasteur Institute, Algeria. The plates were incubated (24 h at 37°C) and resistance was recorded via visual examination. Different antibiotics were tested (Table 2), including ampicillin (AM), amoxicillin (AMX), amoxicillin/clavulanic acid (AMC), oxacillin (OX), penicillin (P), ticarcillin/clavulanic acid (TCC), cefazolin (CZ), cefoxitin (FOX), ceftriaxone (CRO), ceftazidime (CAZ), cefotaxime (CTX), amikacin (AN), kanamycin (K), gentamicin (GM), tobramycin (TM), nalidixic acid (NA), ciprofloxacin (CIP), imipenem (IMP), trimethoprim/sulfamethozole (SXT), clindamycin (DA), colistin (CT), fusidic acid (FA), rifampin (RA), piperacillin (PI), aztreonam (ATM), chloramphenicol (C), streptomycin (S), tetracycline (TE), fosfomycin (FF) and erythromycin (E). Resistance to an antibiotic was confirmed using standard disk diffusion method. Break points to establish resistance were selected based on SFM recommendations for *Enterobacteriaceae* (Bonnet et al., 2010).

RESULTS AND DISCUSSION

Among one hundred analyzed raw cow milk samples, seven were contaminated by *Y. enterocolitica*. Seventeen isolates were identified as *Y. enterocolitica*. Contaminated milk are from five regions: Amieur, Mansourah, Ouled Mimoun, Sebdou and Tlemcen. The greatest number of positive samples was obtained from Ouled Mimoun farms (42.85% with n=7). All the isolates were biotyped by biochemical tests and detection of the virulence genes confirmed their biotype as 1A. The results concerning phenotypic and biochemical characterics of *Y. enterocolitica* strains isolated from west Algerian raw milk are summarized in Table 1.

The thermal death curves at the three treatment temperatures and the D values are shown in Figure 2. The corresponding D_{55}, D_{60} and D_{65} values were 1.34, 0.85 and 0.62 min, respectively. These values were determined experimentally and Figure 3 shows how Z-value was determined. Z value was strongly elevated: 29.98°C.

The raw milk contamination frequency by *Y. enterocolitica* obtained in this study (7%) was higher than that reported in other studies, 1.6% in Iran (Soltan-Dallal et al., 2004) and 1% in Nigeria (Okeke and Okwori, 2014). Therefore, the assessment of *Y. enterocolitica* virulence indicators does not need to be restricted to the detection of plasmid-localized genes of virulence, but requires, at least, one chromosomal virulence-associated gene to be present (Ye et al., 2014). The *ystB* gene is present in all strains of biotype 1A, similar results were also found by Platt-Samoraj et al. (2006) and Jamali et al. (2015). Some researchers believe that these strains *ystB*+ are pathogenic to humans and can cause local outbreaks (Singh and Virdi, 2004b, 2005). The study of Singh and Virdi (2004b) indicated that the *ystB* gene is widely distributed in *Y. enterocolitica* biotype 1A strains and production of *Yersinia* stable toxin Yst-b produced by biotype 1A strains is the major contributor to diarrhea. The presence of *ystB* gene was often associated with clinical cases and represents a risk that should not be ignored. Our results show that it is possible to detect

Table 2. Antibiotic susceptibility of the tested *Y. enterocolitica* strains isolated from Algerian raw milk.

Antibiotics	Disc charge (µg)	*Y. enterocolitica* strains (%) (n=17)		
		S	I	R
Amikacin,	30	100	0	0
Amoxicillin	25	0	0	100
Amoxicillin/Clavulanic acid	30/10	0	0	100
Ampicillin	10	0	11.76	88.24
Aztreonam	30	88.24	11.76	0
Cefazoline	30	0	11.76	88.24
Cefotaxime	30	17.65	35.29	47.06
Cefoxitine	30	29.42	35.29	35.29
Ceftazidime	30	0	41.18	58.82
Ceftriaxone	30	0	5.88	94.12
Chloramphenicol	30	100	0	0
Ciprofloxacin	5	100	0	0
Clindamycin	2	0	0	100
Colistin	10	76.47	0	23.53
Erythromycin	15	0	0	100
Fosfomycin	50	100	0	0
Fusidic acid	10	0	0	100
Gentamicin	10	100	0	0
Impinem	10	100	0	0
Kanamycin,	30	100	0	0
Naldixic acid	30	100	0	0
Oxacillin	5	0	0	100
Penicillin	10	0	0	100
Piperacillin	30	100	0	0
Rifampine	30	17.65	76.47	5.88
Streptomycin	10	100	0	0
Tetracycline	30	100	0	0
Ticarcillin/Clavulanic acid	75/10	0	0	100
Tobramycin	10	100	0	0
Trimethoprim/Sulfamethozol	1.25/23.75	100	0	0

S= Susceptible, I=intermediate, R= resistant, n= number of tested strains.

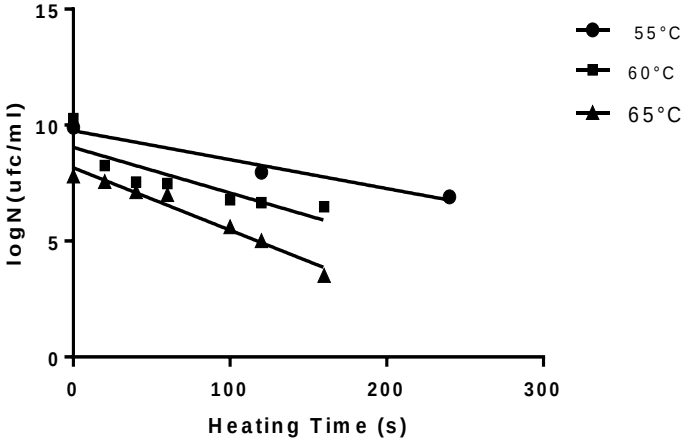

Figure 2. logN versus heating time at different heating temperature: 55°C ●, 60°C ■ and 65°C ▲ for *Y. enterocolitica* YHK261 strain (the experiment was run in duplicate).

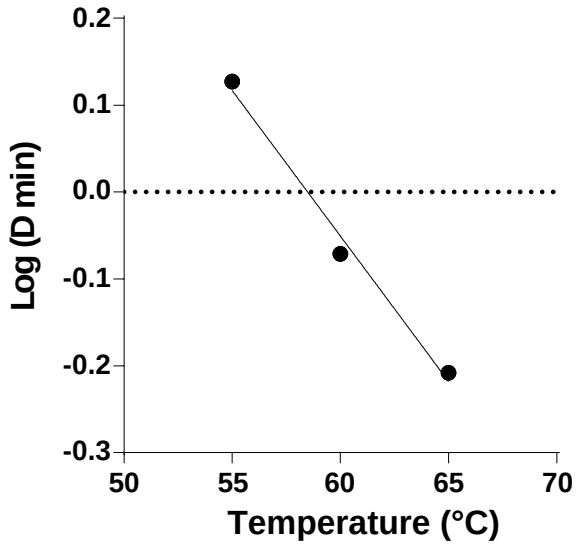

Figure 3. Temperature versus log for *Y. enterocolitica* YHK261 strain (the experiment was run in duplicate).

pathogenic strains with these traits.

Although, there is very little thermal inactivation data published in the scientific literature for *Y. enterocolitica*, Pagán et al. (1999) have reported D_{55} values of 0.33 to 0.78 min and D_{59} values of 0.18 to 0.6 min in citrate phosphate buffer. These values are less than those reported in this paper (1.34 and 0.85 min, respectively). A second study by Bolton et al. (2013) reported D_{55}, D_{60} and D_{65} values of 10.98, 2.53 and 0.60 min, respectively, it is noteworthy that the D_{65} is the same as that obtained. The discrepancies of values may be attributed to the strain variation (Bhagat and Virdi, 2009). This study suggests that minor changes in the temperature of the milk treatment will greatly influence the survival of *Y. enterocolitica* and that mild temperatures are sufficient for the elimination of this microorganism as compared to others such as *Mycobacterium avium subsp. paratuberculosis*, *Coxiella burnetii* and bacterial spores (Pearce et al., 2012). Augmenting the temperature from 55 to 60°C would reduce the D values 1.6 fold, a time-temperature combination of 0.85 min at 60°C is required to achieve one log reduction in *Y. enterocolitica*, the equivalent time at 65°C was 0.62 min.

This study provides D_T and Z values required to eliminate *Y. enterocolitica* and reduce the microbiological risk related to this microorganism without harming the organoleptic and nutritional quality of milk. Outbreaks caused by *Y. enterocolitica* strains have been reported after consumption of pasteurized milk (Ackers et al., 2000) however some heat resistance studies indicate that these strains are unable to survive to the pasteurization suggesting that their presence in pasteurized milk is either due to post-pasteurization contamination or under-processing (Greenwood et al., 1990). *Y. enterocolitica* can multiply at temperatures as low as 4°C (Bottone et al., 1999). Its presence in pasteurized milk, which is generally stored at refrigeration temperatures at the dairy, in the retail chain and at home, may have public health significance.

In Algeria, there is very little research related to food contamination by *Y. enterocolitica.* There are no reports of its presence in raw cow's milk, pasteurized milk, or milk derivatives. Notably, it is usual in this country to prepare dairy products from unpasteurized milk, although this food can be a vehicle of pathogens to humans. This work shows the potential of public health risk in Algeria regarding infections transmitted by raw cow's milk. Therefore, consumers are adviced to mild heat their milk before consumption to avoid the pathogenic *Y. enterocolitica* risk in raw milk or even in pasteurized milk.

Antibiotic susceptibility is of great importance. The results shown in Table 2 indicate that all the tested *Y. enterocolitica* strains (17/17) were susceptible to 13 antibiotics: Amikacin, Chloramphenicol, Ciprofloxacin, Gentamicin, Kanamycin, Naldixic acid, Streptomycin, Fosfomycin, Tetracyclin, Impinem, Piperacillin, Tobramycin and Trimethoprim/Sulfamethozol. Similar patterns of susceptibility were observed among strains isolated from pig tonsils in Switzerland, southern Germany as well as in human strains (Fredriksson-Ahomaa et al., 2009; Bucher et al., 2008). Piperacillin is a representative of ureidopenicillins with a broad scope of antibacterial activity. In the studies of Kot et al. (2008), 77.8% of *Y. enterocolitica* strains were found to belong to biotype 1A.

In veterinary medicine, treatment with antibiotics of penicillin group such as amoxicillin/clavulanic acid, ticarcillin/clavulanic acid, oxacillin, amoxicillin, ampicillin and piperacillin is frequent. The literature cites assessments of *Y. enterocolitica* susceptibility to Amoxicillin, as well as Amoxicillin/Clavulanic acid. In this study, resistance to Ampicillin was shown in all the 17 isolates. Ampicillin showed strong bactericidal activity towards a wide range of microorganisms; however, *Y. enterocolitica* strains are in general resistant to this antibiotic. Ampicillin resistance due to production of β-lactamases is well described in the literature (Bucher et al., 2008). We found no susceptible strains and only 1.94% of strains to be intermediately susceptible to Ampicillin. Singh and Virdi (2004a) found 100% of strains to be resistant, which is also confirmed by results from other authors (Rastawicki et al., 2000). The combination of ampicillin with clavulanic acid significantly broadens the scope of activity and increases the percentage of susceptible strains. In the present study, all strains were fully resistant to amoxicillin/clavulanic acid and ticarcillin/clavulanic acid. The study of Singh and Virdi (2004a) demonstrated that only 2.5% of *Y. enterocolitica* strains were intermediately susceptible,

while the remaining strains were fully resistant to Amoxicillin/Clavulanic acid.

Fifteen strains of 17 (88.24%) are resistant to 1st generation Cephalosporins. This antibiotic group including Cefazolin, are active against Gram-positive and Gram-negative bacteria, while several studies proved that 90% of strains belonging to the *Enterobacteriaceae* family are resistant to Cefazolin (Fredriksson-Ahomaa et al., 2007; Bucher et al, 2008; Bhaduri et al., 2009). Kot et al. (2008) demonstrated that about 90% of biotype 1A *Y. enterocolitica* were susceptible to Cefazolin; these results are not in accordance with our data. Six (35.29%) strains are resistant to 2nd generation cephalosporins including cefoxitin and 6 (35.29%) strains are intermediately sensitive. The 2nd generation Cephalosporins have a stronger activity against Gram-negative than Gram-positive bacteria. Singh and Virdi (2004a) have demonstrated that 41.3 and 37.5%, of *Y. enterocolitica* strains are susceptible and 52.5 and 50% intermediately susceptible, respectively. The third generation Cephalosporins of which Ceftriaxone, Ceftazidime and Cefotaxime were included in earlier studies demonstrated bactericidal activity mainly against *Staphylococcus* sp. and *Streptococcus* sp., but also against *Enterobacteriaceae, Haemophilus influenzae, Borrelia* sp., and *Pasteurella* sp.

Y. enterocolitica are categorized as microorganisms susceptible to the 3rd generation Cephalosporins. In this study, 16, 10 and 8 (94.12, 58.82 and 47.06%) strains show resistance against Ceftriaxon, Ceftazidim and Cefotaxim, respectively. Singh and Virdi (2004a) did not find biotype 1A *Y. enterocolitica* strains resistant to the 3rd generation Cephalosporins. The studies of the Polish clinical strains of *Y. enterocolitica*, serotype O:3, have demonstrated full susceptibility of the strains to the 3rd generation Cephalosporins (Rastawicki et al., 2000). It may be concluded that in this study, the strains of *Y. enterocolitica* isolated from milk varied greatly in terms of their *in vitro* susceptibility to β-lactam antibiotics. *Y. enterocolitica* strains were found to be relatively highly resistant to Cephalosporins and most Penicillins. It is deemed necessary to systematically monitor the *Y. enterocolitica* strains susceptibility to antibiotics.

Due to *Y. enterocolitica* wide spread particularly in dairy product, inappropriate antibiotic treatment and prophylaxis, as well as antibiotics overuse in human and veterinary medicine, may lead to the development of resistant strains to one or several groups of antibiotics (Vose et al., 2001). Bacteria have wide mechanisms to develop antibiotic resistance, therefore it is important to systematically assess their susceptibility to individual antibiotics, thus enabling selection of an optimal treatment and preventing drug resistance spread among bacteria (Caprioli et al., 2000; Fredriksson-Ahomaa et al, 2009). The improper use of antibiotics in the developing countries like Algeria may be the main cause of high resistance rate in local *Yersinia* isolates.

Most importantly, preventive measures such as reasonable antibiotherapy must be adopted to avoid increasing resistance to antibiotics of *Y. enterocolitica*. Moreover using antibiotics as growth promoters must be prohibited.

Conclusion

This study provides data on the occurrence of *Y. enterocolitica* in raw milk in Western Algeria and their resistance ability to antibiotics. The risk due to the presence of *Y. enterocolitica* in raw milk is not insignificant. It highlights the role of raw milk and dairy derivatives as a transmission vehicle of potentially pathogenic *Y. enterocolitica* strains that can transmit antibiotic resistance to the intestinal flora. However, *Y. enterocolitica* is easily eliminated by heat treatment at mild temperatures of about 65°C.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Ackers ML, Schoenfeld S, Markman J, Smith MG, Nicholson MA, DeWitt W, Slutsker L (2000). An outbreak of *Yersinia enterocolitica* O:8 infections associated with pasteurized milk. J. Infect. Dis. 181(5):1834-1837.

AFNOR (2003). Norme NF EN ISO 10273: Microbiologie des aliments : méthode horizontale pour la recherche de *Yersinia enterocolitica* présumées pathogènes. ISO 10273:2003.

Bari ML, Hossain MA, Isshiki K, Ukuku D (2011). Behavior of *Yersinia enterocolitica* in Foods. J. Pathog. 2011(13).

Bernardino-Varo L, Quiñones-Ramírez EI, Fernández FJ, Vázquez-Salinas C (2013). Prevalence of *Yersinia enterocolitica* in raw cow's milk collected from stables of Mexico City. J. Food. Prot. 76(4):694-698.

Bhaduri S, Wesley I, Richards H, Draughon A, Wallace M (2009). Clonality and antibiotic susceptibility of *Yersinia enterocolitica* isolated from US market weight hogs. Foodborne. Pathog. Dis. 6(3):351-356.

Bhagat N, Virdi JS (2010). The Enigma of *Yersinia enterocolitica* biotype 1A. Crit. Rev. Microbiol. 1-15.

Bhagat N, Virdi JS (2009). Molecular and biochemical characterization of urease and survival of *Yersinia enterocolitica* biotype 1A in acidic pH *in vitro.* BMC. Microbiol. 9(1):262.

Bolton DJ, Ivory C, McDowell D (2013). Thermal inactivation of *Yersinia enterocolitica* in pork slaughter plant scald tank water. Meat Sci. 95(3): 668-671.

Bonnet R, Cavallo JD, Chardon H, Chidiac C, Courvalin P, Drugeon H, Dubreuil L, Guery B (2010). Comité de l'Antibiogramme de la Société Française de Microbiologie. CA. SFM. (2010):19-40.

Bottone EJ (1999). *Yersinia enterocolitica*: overview and epidemiologic correlates. Microbes. Infect. 1(4):323-333.

Bucher M, Meyer C, Grötzbach B, Wacheck S, Stolle A, Fredriksson-Ahomaa M (2008). Epidemiological data on pathogenic *Yersinia enterocolitica* in Southern Germany during 2000-2006. Foodborne. Pathog. Dis. 5(3):273-280.

Caprioli A, Busani L, Martel JL, Helmuth R (2000). Monitoring of antibiotic resistance in bacteria of animal origin: epidemiological and microbiological methodologies. Int. J. Antimicrob. Agents. 14(4):295-301.

Darwish SF, Asfour HA, Allam HA (2015). Incidence of *Yersinia*

enterocolitica and *Yersinia pseudotuberculosis* in raw milk samples of different animal species using conventional and molecular methods. A. J. V. S. 44(1):174-185.
European Food Safety Authority (EFSA) (2011). The community summary report on trends and sources of zoonoses, zoonotic agents and foodborne outbreaks in the European Union in 2009. EFSA. J. 9(3):2090.
Falcão JP, Falcão DP, Pitondo-Silva A, Malaspina AC, Brocchi M (2006). Molecular typing and virulence markers of *Yersinia enterocolitica* strains from human, animal and food origins isolated between 1968 and 2000 in Brazil. J. Med. Microbiol. 55(11):1539-1548.
Fredriksson-Ahomaa M, Stolle A, Stephan R (2007). Prevalence of pathogenic *Yersinia enterocolitica* in pigs slaughtered at a Swiss abattoir. Int. J. Food. Microbiol. 119(3):207-212.
Fredriksson-Ahomaa M, Wacheck S, Koenig M, Stolle A, Stephan, R (2009). Prevalence of pathogenic *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* in wild boars in Switzerland. Int. J. Food. Microbiol. 135(3):199-202.
Greenwood MH, Hooper WL, Rodhouse JC (1990). The source of *Yersinia spp.* in pasteurized milk: an investigation at a dairy. Epidemiol. Infect. 104(03):351-360.
Guven A, Sezer C, Aydin BD, Oral NB, Vatansever L (2010). Incidence and pathogenicity of *Yersinia enterocolitica* isolates from foods in Turkey. Kafkas Univ Vet Fak Derg. 16:107-112.
Huovinen E, Sihvonen LM, Virtanen MJ, Haukka K, Siitonen A, Kuusi, M (2010). Symptoms and sources of *Yersinia enterocolitica*-infection: a case-control study. BMC. Infect. Dis. 10(1):122.
ITELV (Institut technique des élevages en Algérie) (2012). Info élevage, bulletin trimestriel n°6. 2012. http://www.itelv.dz/index.php/bulletins-agro-meteo/summary/3-infoselevages/21-infos-elevages-itelv-n6.html (consulted 29 April 2015).
Jamali H, Paydar M, Radmehr B, Ismail S (2015). Prevalence, characterization, and antimicrobial resistance of *Yersinia* species and *Yersinia enterocolitica* isolated from raw milk in farm bulk tanks. J. Dairy. Sci. 98(2):798-803.
Jayarao BM, Henning DR (2001). Prevalence of foodborne pathogens in bulk tank milk. J. Dairy. Sci. 84(10):2157-2162.
Kot B, Jakubczak A, Piechota M (2008). Isolation and characterization of *Yersinia enterocolitica* rods from pig tonsils. Med. Weter. 64(3):283-287.
Lambertz ST, Danielsson-Tham ML (2005). Identification and characterization of pathogenic *Yersinia enterocolitica* isolates by PCR and pulsed-field gel electrophoresis. Appl. Environ. Microb. 71(7):3674-3681.
Mathew AG, Cissell R, Liamthong S (2007). Antibiotic resistance in bacteria associated with food animals: a United States perspective of livestock production. Foodborne Pathog. Dis. 4(2):115-133.
Okeke OFI, Okwori AEJ (2014). Occurrence of pathogenic *Yersinia* species in locally fermented cow milk (nono) in jos Nigeria. N. J. B. 23.
Pagán R, Mañas P, Raso J, Trepat, FJS (1999). Heat resistance of *Yersinia enterocolitica* grown at different temperatures and heated in different media. Int. J. Food. Microbiol. 47(1):59-66.
Pearce LE, Smythe BW, Crawford RA, Oakley E, Hathaway SC, Shepherd, JM (2012). Pasteurization of milk: the heat inactivation kinetics of milk-borne dairy pathogens under commercial-type conditions of turbulent flow. J. Dairy. Sci. 95(1):20-35.
Perkowska K, Platt-Samoraj A, Bancerz-Kisiel A, Szweda W (2011). Susceptibility of Polish *Yersinia enterocolitica* strains isolated from pigs to 12 β- lactam antibiotics. Bull Vet Inst Pulawy. 55:397-402.
Platt-Samoraj A, Ugorski M, Szweda W, Szczerba-Turek A, Wojciech K, Procajło Z (2006). Analysis of the presence of *ail, ystA* and *ystB* genes in *Yersinia enterocolitica* strains isolated from aborting sows and aborted fetuses. J. Vet. Med. 53(7):341-346.
Rahimi E, Sepehri S, Dehkordi FS, Shaygan S, Momtaz H (2014). Prevalence of *Yersinia* Species in Traditional and Commercial Dairy Products in Isfahan Province, Iran. Jundishapur. J. Microbiol. 7(4).
Rastawicki W, Gierczyński R, Jagielski M, Kałużewski S, Jeljaszewicz J (2000). Susceptibility of Polish clinical strains of *Yersinia enterocolitica* serotype O3 to antibiotics. Int. J. Antimicrob. Agents. 13(4):297-300.
Singh I, Virdi JS (2004a). *In vitro* susceptibilities of *Yersinia enterocolitica* biotype 1A. World. J. Microbiol. Biotechnol. 20:329-331.
Singh I, Virdi JS (2004b). Production of *Yersinia* stable toxin (YST) and distribution of *yst* genes in biotype 1A strains of *Yersinia enterocolitica*. Japan. J. Infect. Dis. 53(11):1065-1068.
Singh I, Virdi JS (2005). Interaction of *Yersinia enterocolitica* biotype 1A strains of diverse origin with cultured cells in vitro. Jpn. J. Infect. Dis. 58(1):31-33.
Soltan-Dallal MM, Tabarraie A, Moez Ardalan K (2004). Comparison of four methods for isolation of *Yersinia enterocolitica* from raw and pasteurized milk from Northern Iran. Int. J. Food. Microbiol. 94(1):87-91.
Subha B, Ramakrishnan D, Suganthi V (2009). Antimicrobial resistance pattern of selected *Yersinia enterocolitica* isolated from raw cow milk and pork samples of Namakkal District, Tamilnadu, South India. I. J. E. R. 3(3):169-177.
Tennant SM, Grant TH, Robins-Browne RM (2003). Pathogenicity of *Yersinia enterocolitica* biotype 1A. FEMS. Immunol. Med. Microbiol. 38(2):127-137.
Tudor L, Ţogoe I, Ceauşi C, Mitrănescu E, Goncearov M, Rotaru E (2008). The optimization of *Yersinia enterocolitica* isolation and identification from food products. Lucrari. Stiintifice. USAMV.41:993-1002.
Vose D, Acar J, Anthony F, Franklin A, Gupta R, Nicholls T, Tamura Y, Thompson S, Threlfall EJ, Van Vuuren M, White DG, Costarrica ML (2001). Antimicrobial resistance: risk analysis methodology for the potential impact on public health of antimicrobial resistant bacteria of animal origin. Rev. Sci. Tech. OIE. 20(3):811-827.
Wang X, Cui Z, Wang H, Tang L, Yang J, Gu L, Jin D, Luo L, Qiu H, Xiao Y, Xiong H (2010). Pathogenic strains of *Yersinia enterocolitica* isolated from domestic dogs (Canisfamiliaris) belonging to farmers are of the same subtype as pathogenic *Y. enterocolitica* strains isolated from humans and may be a source of human infection in Jiangsu Province, China. J. Clin. Microbiol. 48(5):1604-1610.
Ye YW, Ling N, Han YJ, Wu QP. (2014). Detection and prevalence of pathogenic *Yersinia enterocolitica* in refrigerated and frozen dairy products by duplex PCR and dot hybridization targeting the *virF* and *ail* genes. J. Dairy. Sci. 97(11): 6785-6791.