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Prevalence, virulence and antibiotic susceptibility of *Salmonella* spp. strains, isolated from beef in Greater Tunis (Tunisia)

Walid Oueslati [a,*], Mohamed Ridha Rjeibi [b], Moez Mhadhbi [b], Mounir Jbeli [c], Samia Zrelli [a], Abdelfettah Ettriqui [a]

[a] Laboratory of Food Microbiology, University Manouba, National School of Veterinary Medicine of Sidi Thabet, 2020 Sidi Thabet, Tunisia
[b] Laboratory of Parasitology, University Manouba, National School of Veterinary Medicine of Sidi Thabet, 2020 Sidi Thabet, Tunisia
[c] Department of National Defense, 1008 Tunis, Tunisia

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ABSTRACT

The aim of this work was to investigate the presence of *Salmonella* spp. in 300 beef meat samples collected from cattle carcasses of different categories (young bulls, culled heifers and culled cows). The detection of *Salmonella* spp. was performed by the alternative VIDAS Easy *Salmonella* technique and confirmed by PCR using *Salmonella* specific primers. *Salmonella* serotypes were determined by slide agglutination tests. The resistance to 12 antibiotics was determined by the diffusion method on Mueller-Hinton agar antibiotic discs. The overall contamination rate of beef by *Salmonella* spp. was 5.7% (17/300). This rate varied from naught (0/100) in bulls' meat to 14% (14/100) in culled cows' meat ($p < 0.001$). The prevalence of *Salmonella* spp. was higher in summer and in cattle with digestive disorders: chronic gastroenteritis (6/17), traumatic peritonitis (3/17) and intestinal obstruction (2/17) ($p < 0.0001$). Of the 17 *Salmonella* isolates, 6 serotypes were identified, namely *Salmonella* Montevideo (8/17), *Salmonella* Anatum (3/17), *Salmonella* Minnesota (2/17), *Salmonella* Amsterdam (2/17), *Salmonella* Kentucky (1/17) and *Salmonella* Brandenburg (1/17) ($p < 0.05$). Unlike other serotypes, *S.* Montevideo was present during the whole year except winter. Almost all of the strains (16/17) were resistant to at least one of the 12 tested antibiotics. Multidrug-resistance concerned 14/17 of the strains, including Amoxicillin (13/17), Tetracycline (12/17), Streptomycin (10/17) and Nalidixic acid (6/17). All the strains were sensitive to the association (Amoxicillin + Clavulanic acid), Cefoxitin and Ceftazidime. In addition, our study showed that all *Salmonella* strains (17) were positive for invasion gene *invA* and negative for the virulence gene *spvC*. Only one isolate (*S.* Kentucky) harbored the *h-li* virulence gene.

1. Introduction

Salmonellosis is a bacterial disease with a rising prevalence in the cattle industry. It is frequent in calves of one to ten weeks age, but also reported in dairy and beef cattle (Randall, Cooles, Osborn, Piddock, & Woodward, 2004). Typical clinical signs of acute *Salmonella* infection include fever associated to diarrhea. Infected animals can be asymptomatic and shed to one billion *Salmonella* a day in their feces (Millemann, 1998). Salmonellosis is a major food-borne disease worldwide (WHO, 2013). The pathogenicity of *Salmonella* depends essentially on its chromosomal and/or plasmatic virulence genes (Okamoto, Filho, & Rocha, 2009; Montero, Herrero, & Rodicio, 2012). The chromosomal *invA* gene enables the invasion of epithelial cells (Galan & Curtiss, 1989). *H-li* gene is involved in the control of phase change and motility of *Salmonella*. In addition, *h-li* gene is required for optimal transcription of several genes of invasion (Millemann, 1998). The virulence plasmid *spv* genes play a role in multiplication of *Salmonella* in its host cell (Rotger & Casadesus, 1999), increase the severity of enteritis. It allows both infection and persistence in extra-intestinal sites (Libby, Lesnick, & Guiney, 2000) and under hostile conditions (Valone & Muller, 1993).

Ciprofloxacin and Cefotaxime are the most commonly used antibiotics for the treatment of invasive *Salmonella* infections in humans (Su & Ou, 2004; Bertrand, Weill, & Vrints, 2006; Whichard, Gay, & Cooper, 2007). However, multidrug resistant *Salmonella* strains are becoming a real worldwide threat (Weill, Grimont, & Cloeckaert, 2006; Bouchrif, Ennajii, & Timinouni, 2008). Since 2002 in Europe an emergence of ciprofloxacin-resistant *Salmonella* spp. isolates has been reported in travelers returning from Northeast and Eastern Africa (Collard, Place, & Denis, 2007). In African countries, multidrug-resistant *Salmonella* spp. strains exhibiting resistance to Ciprofloxacin have been reported in several studies (Aragaw, Molla, Muckle, & Poppe, 2007; Bouchrif et al.,

* Corresponding author.
E-mail address: drwalid04@yahoo.fr (W. Oueslati).

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Table 1
Characteristics of *Salmonella* spp. positive animals and corresponding virulence and antibiotic resistance profile.

Serotype	Animals' ages (years)	Culling motif	Virulence gene			Antibiotic resistance profile											
			invA	*spvC*	*h-li*	1	2	3	4	5	6	7	8	9	10	11	12
Salmonella Montevideo	5	Chronic gastroenteritis	+	-	-	R	S	S	S	S	S	S	R	S	S	R	R
	6	Chronic gastroenteritis	+	-	-	R	S	S	S	S	S	S	S	S	S	R	R
	6	Chronic gastroenteritis	+	-	-	R	S	S	S	S	S	S	S	S	S	R	R
	3	Digestive occlusive syndrome	+	-	-	R	S	S	S	S	S	S	S	S	S	R	R
	6	Chronic lameness	+	-	-	R	S	S	S	S	S	S	S	S	S	R	R
	7	Chronic lameness	+	-	-	R	S	S	S	S	S	S	S	S	S	R	R
	7	Chronic lameness	+	-	-	R	S	S	S	S	S	S	S	S	S	R	R
	10	Chronic mastitis	+	-	-	R	S	S	S	S	S	S	S	S	S	R	R
Salmonella Anatum	3	Chronic gastroenteritis	+	-	-	R	S	S	S	S	S	R	S	R	R	S	S
	6	Traumatic peritonitis	+	-	-	S	S	S	S	S	S	R	S	R	R	S	S
	6	Chronic lameness	+	-	-	R	S	S	R	S	S	R	R	R	S	S	S
Salmonella Minnesota	6	Chronic gastroenteritis	+	-	-	S	S	S	S	S	S	S	S	S	S	S	S
	6	Traumatic peritonitis	+	-	-	S	S	S	S	S	S	S	S	S	S	S	R
Salmonella Amsterdam	3	Traumatic peritonitis	+	-	-	R	S	S	S	S	S	R	S	R	R	S	R
	6	Digestive occlusive syndrome	+	-	-	R	S	S	S	S	S	S	R	S	S	R	R
Salmonella Kentucky	5	Chronic gastroenteritis	+	-	-	R	S	R	R	S	S	R	R	R	R	R	R
Salmonella Brandenburg	9	Chronic mastitis	+	-	-	S	S	S	S	S	S	S	S	S	R	S	S

S: Sensitive; R: Resistant

1: Amoxicillin; 2: Amoxicillin + Clavulanic acid; 3: Chloramphenicol; 4: Cephalothin; 5: Cefoxitin; 6: Ceftazidime.
7: Ciprofloxacin; 8: Gentamicin; 9: Kanamycin; 10: Nalidixic acid; 11: Streptomycin; 12: Tetracycline.

2008; Molla, Berchau, & Kleer, 2006). For these reasons, food-borne diseases caused by *Salmonella* spp. are a significant public health concern around the world (Sire & Garin, 2008; Marrero-Ortiz, Han, & Lynne, 2012). Non-typhoid serovars are increasing in importance as significant pathogens of both humans and animals (Mukhopadhyay & Ramaswamy, 2012). Indeed, there are about 17 million cases of acute gastroenteritis or diarrhea annually due to non-typhoid salmonellosis causing 3 million deaths (Rabsch, Tschäpe, & Baumler, 2001). Over 1 million cases of salmonellosis are attributed to consumption of contaminated foods (meat, eggs…) each year in the United States of America. The most common serotypes associated with human diseases in the U.S.A. are *S.* Typhimurium, Enteritidis, Newport, Heidelberg and Javiana (Scallan et al., 2011). *Salmonella* infections lead to approximately 19,000 cases of hospitalizations and nearly 400 deaths each year in the U.S.A. (Scallan et al., 2011), with an economic burden due to lost work, increased medical costs and deaths (Scharff, 2010). Undercooked beef is the most involved food in gastroenteritis, after eggs and egg products (ANSES, 2011). In France, 35% of food-borne *Salmonella* infections were attributable to the consumption of undercooked beef mainly in children under fifteen (Gauchard, Brisabois, & Espie, 2002). In Tunisia, *Salmonella* food-borne infections constitute an emerging public health problem (Ben Aissa, Troudi, & Belhadj, 2007). The most frequently isolated serotypes, in 2010, were *S.* Enteritidis (45%) and *S.* Typhimurium (27%) (R.T.S.R.B.A, 2012). In 2011, two episodes of *Salmonella* food-borne infections occurred in South Tunisia following the consumption of beef; it caused one death and more than 117 clinical cases (Hamza, 2013).

The deep contamination of meat can be related to either clinical or asymptomatic infections (Vieria-Pinto & Tenreiro, 2012). This phenomenon is increased by the slaughtering stress, especially in the absence of respect of water diet and the animals' rest (Radostits, Blood, & Hinchcliff, 2000). However, surface meat contamination occurs also during the slaughtering from the intestinal contents (Buncic & Sofos, 2012). This fecal contamination is due to poor hygiene during animal slaughtering. There is a high risk of beef meat deep contamination by *Salmonella* spp. especially in sanitary culled animals (Labadie, 1999). The present work aimed to on the one hand estimate the prevalence of *Salmonella* meat deep contamination in both healthy bulls and culled females (heifers and cows) and on the other hand study the isolated *Salmonella* strains.

2. Materials and methods

2.1. Samples

The present study was carried out during the four seasons from September 2013 to August 2014; it concerned a total number of 300 beef meat samples designated to human consumption collected in Greater Tunis (Tunisia) from carcasses. During each season, 75 animals (25 bulls, 25 culled heifers and 25 culled cows) were sampled.

Information about age, sex, disease history and cause of culling of the animals was collected. But, there is no information about the health care provided to the animals in their respective farms. Bulls were healthy at the day of slaughtering but culled females were affected by at least one

disease (chronic gastroenteritis, traumatic peritonitis, intestinal obstruction, chronic feet diseases, chronic mastitis…) (Table 1).

Disposable sterile bags were used to collect approximately 100 g of meat from the inner parts of *iliopsoas* muscle. The meat surface was sterilized and the sample was transferred to a sterile stomacher bag.

As recommended by *Codex Alimentarius* (1987), samples were stored at −18 °C until used in the Laboratory of Food Microbiology National School Veterinary of Medicine, Sidi Thabet (Tunisia).

2.2. Salmonella spp. VIDAS detection

The detection of *Salmonella* spp. by alternative VIDAS Easy *Salmonella* (bioMérieux, SA Marcy l'Étoile, France) technique, was performed in four steps: primary enrichment, selective enrichment, VIDAS and confirmation (Cheung & Kam, 2012). Twenty five grams of meat sample were homogenized in 225 ml of Buffered Peptone Water (Biokar Diagnostics, Beauvais, France). The mixture was incubated at 37 °C for 22 h. A volume of 100 μl of media was transferred to 10 ml *Salmonella* Xpress broth 2 (SX2) (bioMérieux SA, Marcy l'Étoile, France) and incubated at 41.5 °C for 24 h. One milliliter of the culture was heated for 15 min at 100 °C then cooled at room temperature. A volume of 0.5 ml SX2 broth was transferred into a SLM array and deposited in the VIDAS (bioMérieux SA, Marcy l'Étoile, France). Confirmation of positive samples was performed by isolation of *Salmonella* from unheated SX2 broth on selective agar (XLD and SS) (Biokar Diagnostics, Beauvais, France) and identification by urease test followed by API 20E system test (bioMérieux SA, Marcy l'Étoile, France) according to ISO 6579, 2002 reference technique.

2.3. Serotypes of Salmonella strains

Serotypes of *Salmonella* isolates were determined by slide agglutination tests with specific immune sera against *O*, *H* and *Vi Salmonella* antigens (BioRad, Marne-La-Coquette, France) at the National Centre for *Salmonella*, *Shigella* and *Vibrio*, Pasteur Institute of Tunis (Tunisia). The interpretation of the results was performed according to the White–Kauffmann–Le Minor scheme (Popoff, Bockemühl, & Gheesling, 2001).

2.4. Molecular study

InnuPREP DNA Mini Kit (Biometra, Les Ulis, France) was used for the extraction of *Salmonella* genomic DNA from the selective enrichment SX2 broth (bioMérieux SA, Marcy l'Étoile, France): one milliliter of SX2 broth was centrifuged for 10 min at 7500 rpm, the pellet was mixed with a washing solution and proteinase K (20 mg/ml). The mixture was heated at 50 °C for 10 min then centrifuged at 12,000 rpm for 2 min. The pellet was washed twice and DNA was eluted by a centrifugation at 8000 rpm for 1 min then stored at −20 °C until used. The molecular confirmation of *Salmonella* was carried out by performing a PCR detecting a 1 kb DNA fragment (Karraouan, Fassouane, El Ossmani, Cohen, & Bouchrif, 2010), using specific primers: F-5′ACCACGCTCTTTCGTCTGG3′ and R-5GAACTGACTACGTAGACGCTC3′ (Abouzeed, Hariharana, Poppe, & Kibengea, 2000). The invasion gene *invA* and virulence genes *spvC* and *h-li* respectively have sizes of 275, 669 and 173 bp (Karraouan et al., 2010). All *Salmonella* isolated strains were tested for three invasion and virulence genes (*invA*, *h-li* and *spvC*) using three specific primer sets: F-5′TATCGCCACGTTCGGGCAA3′ and R-5′TCGCACCGTCAAAGGAACC3′, F-5′AGCCTCGGCTACTGGTCTTG 3′ and R-5′CCGCAGCAAGAGTCACCTCA3′, F-5′CGGAAATACCATCAAA TA3′ and R-5′CCCAAACCCATACTTACTCTG3′ respectively (Abouzeed et al., 2000).

The PCR mixture consisted of 2.5 μl of 10× PCR buffer (20 mM Tris–HCl; pH 8.5; 50 mM KCl), 3 mM $MgCl_2$, 0.4 mM of each dNTP, 25 μM of each primer, 1.25 U Taq Polymerase (Vivantis, Oceanside, USA), sterile distilled water and 2.5 μl of DNA template.

The DNA amplification was performed using the following program: 1 min denaturation at 95 °C, followed by 35 cycles (95 °C for 20 s, 55 °C for 20 s and 72 °C for 2 min) and a final extension at 72 °C for 4 min (Nayak, Stewart, & Wang, 2004). Amplification was performed in a thermocycler (Esco Swift Max Pro, Horsham PA, USA).

PCR products were electrophorized in 1.5% agarose gel and visualized with ultraviolet transillumination after ethidium bromide staining. Negative and positive controls were added for each PCR run.

2.5. Antibiotic resistance

Resistance test to 12 antibiotics was screened by the diffusion method on Mueller-Hinton agar antibiotic discs (BioRad, Marne-La-Coquette, France). Interpretation of the results was done according to the Clinical and Laboratory of Standards Institute criteria (CLSI, 2008).

2.6. Statistical analyses

The infection prevalence percentages were compared using Epi Info 6 (Dean et al., 2011) with Mantel Haenszel Chi-square test. Observed differences were considered significant when the p value was lower than 0.05 (Schwartz, 1993).

2.7. Ethics statement

The study was performed in accordance with the Memorandum of the French General Directorate of Food No 2012-8056 March 13, 2012 on the authorization of slaughterhouses to derogate from the requirement to stun animals.

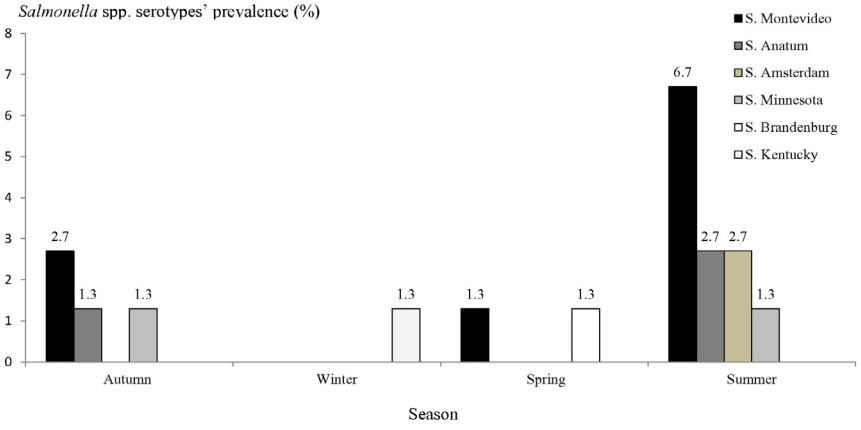

Fig. 1. Seasonal distribution of *Salmonella* spp. serotypes.

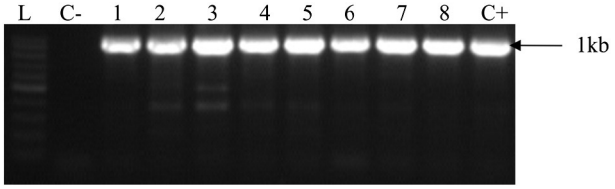

Fig. 2. Agarose gel electrophoresis of bands generated by simple PCR of strains using the primer specific for the genus *Salmonella* (1 kb). L: 100 bp ladder; C−: negative control; C+: positive control; Lanes 1 to 8: *Salmonella* bands (1 kb) of isolated strains.

The experimental protocol was approved by the Tunisian Association of Laboratory Animal Science, Tunisia. The restraint and slaughter of animals were monitored and carried out by trained and competent staff, in order to minimize animals' suffering.

All the stages were realized under the responsibility and supervision of an official sanitary veterinarian.

3. Results

3.1. Prevalence of Salmonella spp. in beef

The deep meat contamination prevalence by *Salmonella* spp. was 5.7% (17/300). The 17 isolates were positive to PCR using *Salmonella* specific primers (Fig. 1). This rate varies from naught (0/100) for bulls' meat to 14% (14/100) for culled cows' meat ($p < 0.001$). *Salmonella* spp. beef meat prevalence was higher during the summer season (13.3%; 10/75) compared to winter (1.3%; 1/75) ($p < 0.05$).

A total number of 6 serotypes were identified, namely *S.* Montevideo (8/17), *S.* Anatum (3/17), *S.* Minnesota (2/17), *S.* Amsterdam (2/17), *S.* Kentucky (1/17) and *S.* Brandenburg (1/17) ($p < 0.05$) (Table 1). Unlike other serotypes, *S.* Montevideo was present during the whole year except winter. It showed a prevalence peak during the summer season (6.7%; 5/75) ($p < 0.05$) (Fig. 1).

Digestive disorders (chronic gastroenteritis (6/17), traumatic peritonitis (3/17) and intestinal obstruction (2/17)) were significantly related to *Salmonella* spp. meat contamination ($p < 0.0001$) (Table 1). Moreover, chronic feet diseases (4/17) and chronic mastitis (2/17) were factors for deep meat *Salmonella* spp. contamination.

Prevalence of *Salmonella* contamination was significantly higher in culled cows' meat with digestive disorders (23.5%; 8/34) compared to cows with chronic feet diseases (12.1%; 4/33) and cows with chronic mastitis (6.1%; 2/33) ($p < 0.001$).

3.2. Prevalence of pathogenic genes and antibiotic resistance

Salmonella strains (17) were positive for invasion gene *invA* and negative for the virulence gene *spvC*. Only one isolate (*S.* Kentucky) serotype was positive for *h-li* gene (Table 1, Fig. 3).

Almost all (16/17) of the strains were resistant to at least one of the antibiotics. Multi-resistance concerned 14/17 of the strains, including Amoxicillin (13/17), Tetracycline (12/17), Streptomycin (10/17) and Nalidixic acid (6/17). Furthermore, all strains were sensitive to the association (Amoxicillin + Clavulanic acid), Cefoxitin and Ceftazidime (Table 1).

4. Discussion

The results of our study showed that *Salmonella* spp. beef contamination rate was 5.7% (17/300). It varies from naught (0/100) in bulls' meat to 14% (14/100) in culled cows' meat ($p < 0.001$). Our results were comparable to those of Ben Jaafar, Jiridi, Fodha, and Salem (2002) who found a prevalence of 4.2% in Tunisian beef meat catering. This rate was lower to the results of Derouiche (2001) in Tunisia (9.3%). This difference could be explained by the fact that Derouiche collected samples from culled cattle.

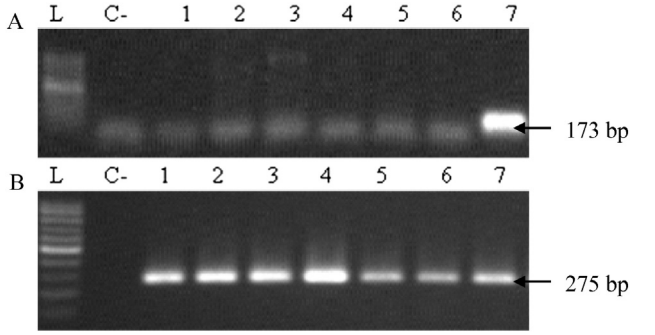

Fig. 3. Agarose gel electrophoresis of *h-li* and *invA* genes products. L: 100 bp ladder; C−: negative control; Lanes 1 to 7: positive samples (*h-li*, *invA*); A panel: *h-li* positive samples; B panel: *invA* positive samples.

Our study revealed that the contamination prevalence of the culled cows' meat (14%) was significantly higher than that of culled heifers and bulls' meat (3% and 0% respectively) ($p < 0.001$) (Table 1). This is likely due to the high prevalence of latent carriage in culled cows, immunodepression in some animals, the absence of water diet and fatigue before slaughtering (Labadie, 1999).

Seasonal fluctuation of *Salmonella* spp. beef meat contamination showed a significant difference between the prevalence of *Salmonella* spp. during the summer season (13.3%; 10/75) and winter (1.3%; 1/75) ($p < 0.05$). Our results were comparable to those of Ayaz, Ormeci, and Oz (2010) who found a higher prevalence of *Salmonella* spp. in Turkish beef meat in summer than in other seasons. In Nepal, Maharjan, Vandana, Durga, and Poornima (2006) indicated that the seasonal prevalence of *Salmonella* spp. in beef meat was highest in the months of April and May (summer season).

Of the 17 *Salmonella* isolates, 6 serotypes were identified, namely, *S.* Montevideo (8/17), *S.* Anatum (3/17), *S.* Minnesota (2/17), *S.* Amsterdam (2/17), *S.* Kentucky (1/17) and *S.* Brandenburg (1/17) ($p < 0.05$) (Table 1). Seasonal distribution of *Salmonella* spp. serotypes indicated that unlike other serotypes, *S.* Montevideo was present during the whole year except winter and peaking during the summer season (6.7%; 5/75) ($p < 0.05$) (Fig. 1). *S.* Montevideo is frequently isolated from diarrhea cattle in France (AFSSA, 2006). Since 2000, *S.* Montevideo was the third most frequent serotype in cattle digestive diseases in France (7%) (AFSSA, 2006). However, our results do not agree with those of Derouiche (2001) and Ben Jaafar et al. (2002) who revealed the predominance of *S.* Anatum (40%) followed by *S.* Corvallis. *S.* Montevideo rate was 6% (Derouiche, 2001) but not isolated by Ben Jaafar et al. (2002).

Our study did not reveal the presence of the two most frequent serotypes involved in collective food poisoning namely *S.* Enteritidis and *S.* Typhimurium. Ben Jaafar et al. (2002) showed fairly significant levels of contamination of beef with *S.* Enteritidis and *S.* Typhimurium (12%) but Derouiche (2001) indicated a low level of contamination with *S.* Enteritidis (4%) but no *S.* Typhimurium contamination was found.

Digestive disorders (chronic gastroenteritis (6/17), traumatic peritonitis (3/17) and intestinal obstruction (2/17)) were the main cattle disease history (Table 1). This is explained by the fact that digestive tract disturbances increase *Salmonella* absorption from the intestinal lumen (Labadie, 1999).

Our study showed that all studied *Salmonella* strains ($n = 17$) were positive for invasion gene *invA* and negative for *spvC* virulence gene. Only one isolate (*S.* Kentucky) was positive for *h-li* gene (Table 1, Figs. 2 and 3). Our results were similar to those of Karraouan et al. (2010) who indicated that all strains ($n = 39$) of *Salmonella* were positive for the invasion gene *invA*. Several serotypes (Muenster, Newport, Corvallis, Albert, Bredeney, Hadar, Derby, Kiel, Altona and Infantis) were negative for both virulence genes *spvC* and h-li, while 4/8 of *S.* Kentucky serotype were positive for the gene *h-li*. Only *S.* Gallinarum was positive

for the *spvC* virulence gene. Abouzeed et al. (2000) reported similar results about the *invA* gene in 75 *Salmonella* strains from turkey meat, bovine and human samples.

PCR analysis demonstrated the *h-li* gene in only one of the 17 isolates. Karraouan et al. (2010) showed that 18% (7/39) of strains possess the *h-li* gene. The results of our study about the presence of the gene *spvC* are not consistent with those of Abouzeed et al. (2000); they revealed a frequency of 28% *spvC* gene (21/75) among different serotypes of *Salmonella* isolated from turkey meat, cattle and human disease samples. Our results were similar to those of Turki, Ouzari, and Ben Aissa (2012) who indicated that all strains (n = 57) of *S.* Kentucky isolated from different sources (animals, food and human cases) were negative for the *spvC* gene.

As it was reported in several countries (France, Belgium, Slovak Republic, Morocco, and Ethiopia) (Aragaw et al., 2007; Collard et al., 2007; Molla et al., 2006), we noticed a high multidrug-resistance rate in *Salmonella* isolated from beef.

Our study indicated that almost all (16/17) of the strains were resistant to at least one of the 12 tested antibiotics. Multidrug-resistant *Salmonella* concerned 14/17 of the strains, including Amoxicillin (13/17), Tetracycline (12/17), Streptomycin (10/17) and Nalidixic acid (6/17). In Tunisia, to our knowledge there is no published data on antimicrobial use in farm animals. In Tunisian human medicine practice, an antibacterial treatment is not indicated for uncomplicated forms of *Salmonella* gastroenteritis. Quinolone therapy is reserved to clinically severe cases (Boutiba et al., 2007).

Our results are similar to those from the study of Karraouan et al. (2010) which studied antibiotic resistance of 39 *Salmonella* isolates from turkey meat in Morocco. This study showed that 82% (32/39) of the strains were resistant to at least one of the tested antibiotics, while the multidrug-resistant strains were present in 51.3% of the samples. High percentages of resistance were observed for Sulfonamides (64%), Tetracycline (39%), Amoxicillin (31%) and Nalidixic acid (36%). The rates of resistance against Amoxicillin and Tetracycline were high in Tunisian hospitals (54.5 and 40% respectively) (Boutiba et al., 2007).

Furthermore, our study showed that all the strains were sensitive to Amoxicillin and Clavulanic acid association and Cefoxitin. But, Boutiba et al. (2007) reported a high rate of resistance against third generation cephalosporin in Tunisian hospitals (33%). *S.* Kentucky was the most resistant serotype (9/12). Similar results were reported by Karraouan et al. (2010).

5. Conclusion

Our study revealed a low prevalence of *Salmonella* spp. meat contamination. This rate varied from naught for bulls meat to 14% for culled cows' meat. In addition, the prevalence of *Salmonella* spp. was higher in summer and in cattle with digestive disorders. Six serotypes of *Salmonella* were identified; *S.* Montevideo was the most frequent, followed by *S.* Anatum, *S.* Minnesota, *S.* Amsterdam, *S.* Kentucky and *S.* Brandenburg. Unlike other serotypes, *S.* Montevideo was present during the whole year except winter. All *Salmonella* strains were positive for invasion gene *invA* and negative for *spvC* virulence gene. Only one (*S.* Kentucky) was positive for *h-li* gene. Almost all of the strains were resistant to at least one antibiotic. Multidrug-resistant *Salmonella* concerned many serotypes, including Amoxicillin, Tetracycline, Streptomycin and Nalidixic acid. *S.* Kentucky was the most resistant serotype. The association of multidrug-resistance and invasion gene in *Salmonella* isolates justifies the need of surveillance system for both human and animal health sectors.

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