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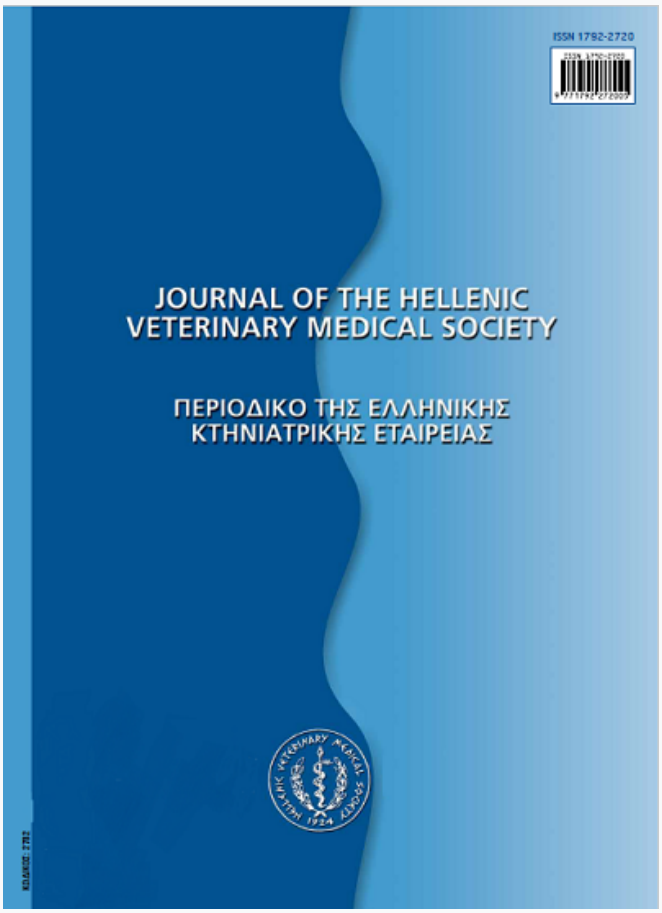

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Research article
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Prevalence and antimicrobial resistance of *Salmonella* isolated from bovine and ovine samples in slaughterhouses of Algiers, Algeria

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ABSTRACT. The aims of our work are to estimate the prevalence of Salmonella isolated from carcasses and feces of cattle and sheep in the two biggest slaughterhouses in Algiers, Algeria, and to characterize the obtained strains by serotyping and antimicrobial resistance testing. The detection of *Salmonella* was performed by the conventional culture method and isolates were confirmed by PCR. Susceptibility to antibiotics was carried out by agar disc diffusion method. The results showed that 10.17% of samples were *Salmonella* positive. Carcass samples were more contaminated than fecal samples. Serotyping of the 84 *Salmonella* isolates has enabled to identify 10 different serovars; the most predominant was *S.* Muenster. The invA gene was detected in 96.43% of isolates whereas all *S.* Typhimurium strains were positive for spy gene. Sixty-eight (80.95%) isolates were resistant to at least one of the 28 antibiotics tested and exhibited 17 different antimicrobial resistance patterns. The most frequently observed resistance was to streptomycin (69.05%). While 22.62 % of the isolates were MDR, two *S.* Typhimurium showed an"ACSSuT" pentaresistance pattern. Considering the importance of this group of bacteria for public health, *Salmonella* control is necessary at several steps of food production to ensure safe products for consumers.

Keywords: *Salmonella*, slaughterhouse, prevalence, serovars, antimicrobial resistance.

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INTRODUCTION

The genus *Salmonella* is a Gram negative rod-shaped bacteria belonged to the family of *Enterobacteriaceae*. It includes more than 2500 serovars that inhabit the gastrointestinal tracts of various domestic and wild animal species (Bahnass et al., 2015). It is estimated that salmonellosis represents 93.8 (16%) million cases of human gastroenteritis among an estimated 582 million cases of 22 different food borne enteric diseases, and it is responsible for 155 000 deaths worldwide each year (Elgroud et al., 2015; Manoj et al., 2015). *Salmonella enterica* subsp. *enterica* serovar Typhimurium (*S.* Typhimurium) is the causative agent of 46% of outbreaks (Ahmed and Shimamoto, 2014). The most common contaminated foods associated with human salmonellosis are poultry, beef, eggs, seafood, and dairy products (Abbassi-Ghozzi et al., 2012). At the production level, inadequate sanitation in slaughterhouses, improper handling of meat, and cross- contamination through processing equipment are some of the most frequent causes of the spread of *Salmonella* (Singh and Mustapha, 2014).

Conventional bacterial culture methods are still used most often to detect and identify *Salmonella* and require at least 3-11 days including selective enrichment and plating followed by biochemical tests (Karmi, 2013). Recently, PCR-based techniques are used effectively for rapid detection of *Salmonella* serovars (Can et al., 2014).

The purposes of this study are to monitor the prevalence of *Salmonella* in bovine and ovine carcasses and feces in Algiers, by using conventional culture method and PCR assay, and to determine the antimicrobial resistance profiles of the isolates. Bovine and ovine carcasses were used as test items because they are widely consumed in Algeria. Additionally, a few national studies have been conducted on the prevalence of *Salmonella* in red meats.

MATERIAL AND METHODS

Samples collection

Cattle and sheep were brought to El-Harrach and Hussein Dey slaughterhouses from different regions of the country. During two periods, from February to June 2013, and from December 2013 to May 2014, 826 ovine and bovine samples were collected among which 190 from bovine carcasses, 251 from ovine carcasses, 160 bovine feces and 225 from ovine feces. Samples were obtained immediately after evisceration. Carcass samples were obtained using the wet and dry swabbing method with 2 sponges for each of the four sites chosen in accordance with Annex A of the ISO standard 176048. The four wet-dry swabs pairs from each carcass were pooled, and processed as one sample. Fresh fecal samples were collected directly from rectum at the time of slaughtering and packed into separate sterile polyethylene bags. Then, all samples were transported on ice to the laboratory for immediate processing and analysis.

Isolation and identification of *Salmonella* spp. by conventional method

Isolation of *Salmonella* spp. from all samples was performed according to the ISO norm 6579:2002 (Annex D010705). Briefly, fecal samples were diluted at 10-1 with buffered peptone water (Institut Pasteur d'Algérie [IPA], Algiers, Algeria). Carcass swabs were put into 100 mL BPW.

After incubation, 1 and 0.1mL of pre-enriched broth were, respectively, transferred to Müller Kauffmann Tetrathionate- novobiocin broth (IPA, Algiers, Algeria) and to Rappaport-Vassiliadis with soya (IPA, Algiers, Algeria), then incubated for 24 h at 37 °C and 42°C, respectively. A loopful from each selective enrichment broth was streaked onto selective xylose-lysine-deoxycholate and Hektoen agar plates (IPA, Algiers, Algeria), and incubated at 37°C for 24 h. Presumptive *Salmonella* colonies were characterized using classical biochemical tests with Triple Sugar Iron (TSI; IPA) agar slant, indole urea reagent (IPA), Lysine Decarboxylase (LDC; IPA) reagent and *ortho*-NitroPhenyl-β-galactoside (ONPG; IPA) , as well as Api 20E gallery (Biomérieux, SA, France).

Serotyping was carried out using slide agglutination with commercial poly O (OMA, OMB), and poly H antigen specific antisera (Difco, Sparks, MD. USA). Once the antigenic formulae were obtained, the Kauffmann-White scheme was used to name the serovars.

Molecular confirmation

Molecular confirmation of isolated *Salmonella*

strains was carried out using the PCR technique. *S.* Typhimurium ATCC 14028 was used as positive control in this study.

DNA extraction

The genomic DNA was extracted from a culture incubated overnight. One or two colonies were suspended in 500 µL of molecular biology water (AccuGENE®, Lonza Group Ltd., Basel, Switzerland), and boiled 10 minutes at 95°C. After adding 100 µL of 5 M NaCl, and centrifuging, the supernatant was removed to a new tube, and 500 µL of cold 100% ethanol were added. A second centrifugation was performed and the supernatant was poured and the DNA pellet was washed in 500 µL of 70% ethanol, and centrifuged, then dried for 30 min at 37°C. Finally, the DNA was re-suspended in 100 µL of DEPC water (Sigma-Aldrich, St. Louis, MO, USA), and stored at - 20°C until use.

Primers sets and PCR amplification

Specific primers for *Salmonella* spp. and *S.* Typhimurium have been published previously (Rahn et al., 1992; Olsen et al., 1995).

Individual PCR assays were performed according to the original published protocols (Rahn et al., 1992; Can et al., 2014). The PCR mixture was consisted of 25 µL final volume containing 1X PCR buffer (Sigma- Aldrich, St-Louis, USA), 200µM of each dNTPs (Dr. Zeydanlı Life Sciences Ltd., Ankara, Turkey), 3mM of MgCl2 (Sigma- Aldrich, St-Louis, USA), 1U *Taq* polymerase (Sigma- Aldrich, St-Louis, USA), 0.4 µM of each primer, and 2 µL template DNA.

The following amplification conditions were used: an initial denaturation step for 3 min at 94°C. Then, 30 cycles, each one consisting of denaturation at 94°C for 30 seconds, 58°C as annealing temperature for 45 seconds, and elongation at 72°C for 60 seconds. Finally, a terminal elongation step of 5 min at 72°C was performed.

For *Salmonella* serovar Typhimurium, PCR protocol was the same, except the annealing temperature, which was established at 55°C (Can et al., 2014).

Electrophoresis of PCR products

The PCR amplified products were electrophoresed in 1.5% agarose gel (AXYGEN Bioscience), stained with 3 µL/g of Ethidium Bromide (Dr. Zeydanlı Life Sciences Ltd., Ankara, Turkey). A 100 pb ladder (AXYGEN Bioscience) was served as a molecular weight marker. In each PCR run, a non-template control (negative control) was included to detect possible external DNA contamination. DNA bands were visualized under UV transillumination (UVP, Upland, USA) and photographed.

Antimicrobial susceptibility test

Antimicrobial susceptibility patterns of *Salmonella* isolates were determined by the agar disk diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) using Mueller- Hinton agar (IPA, Algiers, Algeria). The plates were incubated at 35°C for 24h. The following antibiotic discs (Oxoid, Hampshire, United Kingdom) were used: ampicillin (AMP, 10 µg), ticarcillin (TIC, 75 µg), piperacillin (PRL, 100 µg), amoxicillin (AML, 25 µg), mecillinam (MEL,10 µg), cefazolin (KZ, 30 µg), cefoxitin (FOX, 30 µg), ceftazidine (CAZ, 30 µg), ceftriaxone (CRO, 30 µg), cefepime (FEP, 30 µg), amoxicillin/clavulanate (AMC, 20 µg/10 µg), aztreonam (ATM, 30 µg), imipenem (IPM, 10 µg), kanamycin (K, 30 µg), gentamicin (GM, 10 µg), netilmicin (NET, 30 µg), streptomycin (S, 10 µg), nalidixic acid (NA, 30 µg), norfloxacin (NOR, 10 µg), ciprofloxacin (CIP, 5 µg), sulphonamides (SSS, 300 µg), trimethoprim (W, 5 µg), trimethoprim/sulfamethoxazole (SXT, 1.25 µg/23.75 µg), furans (F, 300 µg), chloramphenicol (C, 30 µg), tetracycline (Te, 30 µg), colistin (CT, 10 µg), and fosfomycin (FOS, 50 µg). The results were recorded by measuring the inhibition zones and scored as sensitive, intermediate, and resistant according to the Clinical Laboratory Standards Institute (CLSI, 2008) criteria.

RESULTS

***Salmonella* prevalence**

Out of 826 samples, 84 (10.17%) were *Salmonella* positive. The relative prevalence within the slaughterhouses was 14.7% (56/381) for El-Harrach and 6.29% (28/ 445) for Hussein Dey. *Salmonella* was detected in all types of samples with different frequencies, 20.52% (39/190) in bovine carcasses, 12.74% (32/251) in ovine carcasses, 6.87% (11/160)

in bovine feces, and 0.89% (2/225) in ovine feces. Bovine samples were more contaminated (14.28%) than ovine samples (7.14%). The frequency of isolation was higher from carcasses (16.10%) than from the feces ones (3.37%) (Table 1).

(14.28%) and *S.* Anatum (13.09%). The highest proportions of *S.* Muenster were recovered from bovine and ovine carcasses, with 48.48% and 33.33%, respectively, compared to bovine and ovine feces (15.15% and 3.03% respectively) (Table 2).

Table 1. Prevalence of *Salmonella* in bovine and sheep carcass and fecal samples from the two slaughterhouses

Animal species	Slaughterhouses								Total	
	El-Harrach				Hussein Dey					
	Carcasses		Feces		Carcasses		Feces			
	n	n Positive (%)	n	n Positive (%)	n	n Positive (%)	n	n Positive (%)	**n**	**n Positive (%)**
Bovine	85	29 (34.12%)	78	9 (11.54%)	105	10 (9.52%)	82	2 (2.44%)	**350**	**50 (14.28%)**
Ovine	112	18 (16.1%)	106	00 (0.0%)	139	14 (10.1%)	119	2 (3.4%)	**476**	**34 (7.14%)**
Total	**197**	**47 (23.86%)**	**184**	**9 (4.89%)**	**244**	**24 (9.84%)**	**201**	**4 (1.99%)**	**826**	**84 (10.17%)**

n: number of samples.

Table 2. Distribution of *Salmonella* serovars.

Serovar	**Slaughterhouses**												**Total (%)**
	El-Harrach						**Hussein Dey**						
	Bv C	Ov C	**Total**	Bv F	Ov F	**Total**	Bv C	Ov C	**Total**	Bv F	Ov F	**Total**	
S. Muenster	14	7	**21**	5	0	**5**	2	4	**6**	0	1	**1**	**33 (39.28)**
S. Kentucky	3	2	**5**	0	0	**0**	6	2	**8**	0	0	**0**	**13 (15.47)**
S. Infantis	4	6	**10**	2	0	**2**	0	0	**0**	0	0	**0**	**12 (14.28)**
S. Anatum	1	0	**1**	0	0	**0**	1	7	**8**	1	1	**2**	**11 (13.09)**
S. Richmond	4	0	**4**	0	0	**0**	0	0	**0**	0	0	**0**	**4 (4.76)**
S. Havana	2	0	**2**	0	0	**0**	1	0	**1**	0	0	**0**	**3 (3.57)**
S. Typhimurium	0	2	**2**	0	0	**0**	0	1	**1**	0	0	**0**	**3 (3.57)**
S. Montevideo	1	0	**1**	2	0	**2**	0	0	**0**	0	0	**0**	**3 (3.57)**
S. Virginia	0	1	**1**	0	0	**0**	0	0	**0**	0	0	**0**	**1 (1.19)**
S.Braenderup	0	0	**0**	0	0	0	0	0	**0**	1	0	**1**	**1 (1.19)**
Total (%)	**29**	**18**	**47 (55.95)**	**9**	**0**	**9 (10.71)**	**10**	**14**	**24 (28.57)**	**2**	**2**	**4 (4.76)**	**84**

Bv C: bovine carcasses, Ov C: ovine carcasses, Bv F: bovine feces, Ov F: ovine feces.

Distribution of *Salmonella* serovars

A position ten (10) different serovars were identified. The most common was *S.* Muenster (39.28%) followed by *S.* Kentucky (15.47%), *S.* Infantis

Molecular confirmation

B position in PCR assay, using S139 and S141 primers belonging to *inv*A gene that amplifies a 284 bp sequence of the *inv*A gene, 96.43% (81/84) of pos-

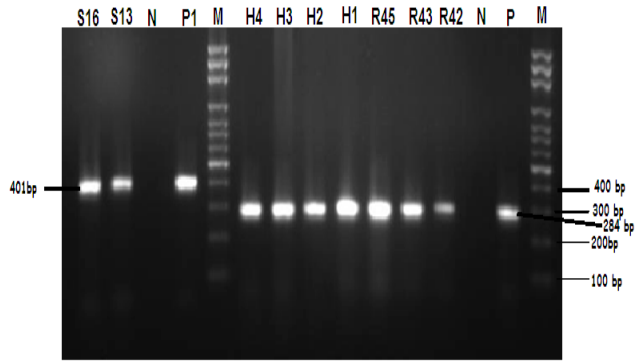

Fig. 1 Specific PCR of *Salmonella* isolates using primer sets *inv*A and *Spy*.
M: 100bp Marker (AXYGEN Bioscience), P: Positive Control (*Salmonella* spp.), N: Negative control (PCR mixture without DNA), R42 R43 R45 H1 H2 H3 and H4: Analyzed isolates showing positive 284 bp DNA fragment of *inv*A gene specific for *Salmonella* spp., P1: Positive control (*Salmonella* Typhimurium), S13and S16: Analyzed isolates showing positive 410bp DNA of *Spy* gene specific for *Salmonella* Typhimurium.

itive samples in conventional culture method including *arizona* generated a single 284 bp amplified DNA fragment on agarose gel (Fig. 1).

Serotyping revealed 3 *S.* Typhimurium isolates of ovine carcass origin and PCR assay shown the presence of specific amplified product 410bp obtained with S. Typhimurium primers chosen from the *Spy* gene (Fig. 1)

Antimicrobial resistance of isolates

From a total of 84 *Salmonella* isolates evaluated for resistance against a panel of 28 selected antimicrobial agents, 68 (80.95%) were resistant to at least one antimicrobial.

All *Salmonella* isolates were susceptible to cefoxitin, ceftazidine, ceftriaxone, cefepime, aztreonam, imipenem, kanamicin, netilmicin, colistin, and fosfomycin. Resistance to the remaining 18 antimicrobials varied between 1.19% and 69.05%. The highest resistance rate observed was to streptomycin (69.05%), followed by sulphonamides (32.14%), then ampicillin, ticarcillin, piperacillin, amoxicillin, nalidixic acid and tetracycline (each 17.86%) (Table 3).

Out of the 68 resistant *Salmonella* isolates, 19 (22.62%) were multidrug resistant (MDR) among which 15 isolates were resistant to more than 5 antimicrobials. Twelve isolates of *S.* Kentucky isolated displayed resistance to at least 10 antimicrobials including fluoroquinolones. The three *S.* Typhimurium strains expressed resistance to more than 9 antimicrobials, including the ‘‘ACSSuT’’ pentaresistance pattern showed by two strains. Among the 10 serovars identified, resistance was found in 9 of them. Only the 3 isolates of *S.* Havana did not show any resistance to all antimicrobials tested. Table 4 displayed 17 different resistance patterns including 14 MDR patterns.

DISCUSSION

Salmonella prevalence

Table 3. Antimicrobial resistance of *Salmonella* isolates from bovine and ovine samples

Serovars	n	Antibiotics*																		Recapitulatory		
		AMP	TIC	PRL	AML	AMC	MEL	KZ	GM	S	SSS	W	SXT	NA	NOR	CIP	C	TE	F	0	1	2-5	+5
S. Muenster	33	0	0	0	0	0	0	0	0	19	8	0	0	0	0	0	0	0	6	27	0	0
S. Anatum	11	0	0	0	0	0	0	0	0	3	2	0	0	0	0	0	0	1	0	7	2	2	0
S. Infantis	12	0	0	0	0	0	0	0	0	12	0	0	0	0	0	0	0	0	0	0	12	0	0
S. Kentucky	13	12	12	12	12	6	4	10	9	13	13	1	1	12	11	12	0	11	0	0	0	1	12
S.Havana	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0
S. Richmond	4	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	4	0	0
S. Typhimurium	3	3	3	3	3	2	0	0	0	3	3	1	1	2	0	0	2	3	1	0	0	0	3
S. Montevideo	3	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	3	0	0
S. Virginia	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0
S. Braenderup	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0
Total	84	15	15	15	15	8	4	10	9	58	27	2	2	15	11	12	2	15	1	16	49	4	15
(%)	100	17.86	17.86	17.86	17.86	9.52	4.76	11.90	10.71	69.05	32.14	2.38	2.38	17.86	13.09	14.28	2.38	17.86	1.19	19.05	58.33	4.76	17.86

*n: number of isolates, AMP: ampicillin, TIC: ticarcillin, PRL: piperacillin, AML: amoxicillin, AMC: amoxicillin/clavulanate, MEL: mecillinam, KZ: cefazolin, GM: gentamicin, S: streptomycin, SSS: sulphonamides, W: trimethoprim, SXT: trimethoprim/sulfamethoxazole, NA: nalidixic acid, NOR: norfloxacin, CIP: ciprofloxacin, C: chloramphenicol, Te: tetracycline, F: furans.*All Salmonella isolates were susceptible to cefoxitin, ceftazidine, ceftriaxone, cefepime, aztreonam, imipenem, kanamicin, netilmicin, colistin, and fosfomycin.*

Table 4. Resistance pattern profiles of isolated *Salmonella* strains.

Salmonella serovars (n/N)	Resistance patterns	n
S. Muenster (27/33)	S	19
	SSS	8
S. Anatum (4/11)	S	2
	S,SSS	1
	SSS,TE	1
S. Infantis (12/12)	S	12
S. Kentucky (13/13)	S,SSS,W,SXT	1
	AMP,TIC,PRL,AML,KZ,S,SSS,NA,CIP,TE	1
	AMP,TIC,PRL,AML,KZ,S,SSS,NA,NOR,CIP	1
	AMP,TIC,PRL,AML,KZ,S,SSS,NA,NOR,CIP,TE	1
	AMP,TIC,PRL,AML,GM,S,SSS,NA,NOR,CIP,TE	1
	AMP,TIC,PRL,AML,KZ,GM,S,SSS,NA,NOR,CIP,TE	1
	AMP,TIC,PRL,AML,MEL,GM,S,SSS,NA,NOR,CIP,TE	1
	AMP,TIC,PRL,AML,AMC,KZ,GM,S,SSS,NA,NOR,CIP,TE	3
	AMP,TIC,PRL,AML,AMC,MEL,KZ,GM,S,SSS,NA,NOR,CIP,TE	3
S. Richmond (4/4)	S	4
S. Typhimurium (3/3)	AMP,TIC,PRL,AML,S,SSS,W,SXT,TE	1
	AMP,TIC,PRL,AML,AMC,S,SSS,NA,C,TE	1
	AMP,TIC,PRL,AML,AMC,S,SSS,NA,C,TE,FU	1
S. Montevideo (3/3)	S	3
S. Virginia (1/1)	NA	1
S. Braenderup (1/1)	S,SSS	1

N: total of isolates, n: number of resistant isolates, AMP: ampicillin, TIC: ticarcillin, PRL: piperacillin, AML: amoxicillin, AMC: amoxicillin/clavulanate, MEL: mecillinam, KZ: cefazolin, GM: gentamicin, S: streptomycin, SSS: sulphonamides, W: trimethoprim, SXT: trimethoprim/sulfamethoxazole, NA: nalidixic acid, NOR: norfloxacin, CIP: ciprofloxacin, C: chloramphenicol, Te: tetracycline, F: furans.

In the current study, out of 826 tested samples, 84 (10.17%) were positive for *Salmonella* reflecting the failure of hygiene practices during the slaughtering. Furthermore, it is suggested that the presence of even small numbers of *Salmonella* species in carcasses may lead to heavy contamination of the finished retail product (Dabassa and Bacha, 2012; Ateba and Mochaiwa, 2014). According to the results shown in table 1, bovine samples were more contaminated (14.28%) than ovine samples (7.14%). This result in agreement with previous Algerian data (Nouichi and Hamdi, 2009; Mezali and Hamdi, 2012), could be due to the particular susceptibility of bovine species to *Salmonella* infection (Nouichi and Hamdi, 2009).

Bovine carcasses are most contaminated by *Salmonella* (20.52%). Previously, we have reported a lower rate of contamination (10%) in the same type of samples (Nouichi and Hamdi, 2009). Worldwide, previous reports indicated a variable prevalence of *Salmonella* in beef meat and carcasses ranged from 1.4% to 13.3% (Dabassa and Bacha, 2012; Tafida et al., 2013; Ahmed and Shimamoto, 2014; Ateba and Mochaiwa, 2014; Dong et al., 2014).

In ovine carcasses, the prevalence was 12.74%. While Teklu and Negussie (2011) registered a similar result (14.1%), other studies reported lower rates of contamination: 1.11% (Nouichi and Hamdi, 2009), and 3.3% (Dabassa and Bacha, 2012).

The prevalence of *Salmonella* in bovine feces was 6.87%. Compared to other studies that evaluated *Salmonella* in cattle fecal samples, our results corroborate relatively the reports of Addis et al. (2011), and Bahnass et al. (2015), who found 7.69% and 8.5%, respectively. Yet, it is more than 7 times lower than the 52% found by Kagambèga et al. (2013). On the other hand, Bordonaro et al. (2015) reported a very low rate (1.7%).

The prevalence of *Salmonella* in ovine fecal samples remains fairly low (0.89%) compared to that

recorded in feces of cattle (7.5%), and that reported in previous studies: 3.3% (Dabassa and Bacha, 2012), and 6.4% (Bahnass et al., 2015).

The present study showed a considerably higher prevalence of *Salmonella* in carcass samples (16.10%) than feces (3.38%), which is consistent with the findings from previous reports of Teklu and Negussie (2011)), Dabassa and Bacha (2012) on sheep and cattle samples, respectively. However, our results contrast with the study's finding of Dong et al. (2014), which indicated higher *Salmonella* prevalence in feces than in carcasses. The relationship between fecal shedding and carcass contamination seems weak. It may be related to the fact that healthy carrier animals especially bovines excrete only a few number of *Salmonella*, unless they undergo some kind of stress (Teklu and Negussie, 2011), and the high level of *Salmonella* on carcasses might be explained by contamination from other sources such as animal skins, operators' hands and equipment, considering the non-respect of slaughtering hygienic rules observed during our study. Nevertheless, the presence of even a carrier animal can be a potential source of contamination of carcasses, environment, material and personnel. Furthermore, fecal samples tend to be less clean than carcasses and other food products samples, and therefore, it is more difficult to grow and detect *Salmonella* in these samples because of other organisms and species of competitive bacteria (Bordonaro et al., 2015).

Distribution of *Salmonella* serovars

Ten serovars were identified in the current study. Based on the results obtained, there seemed to be a difference in the types of *Salmonella* serovars from the different sources: *S.* Muenster and *S.* Anatum were isolated from the four categories of samples. This would likely reflect cross-contamination from multiple sources and poor hygiene conditions in the slaughterhouses. While certain serovars were exclusively recovered from only one source, such as, *S.* Richmond and *S.* Typhimurium, which were isolated from cattle and sheep carcasses, respectively. The identification of *S.* Kentucky only in carcass samples could be explained that the origin of the contamination wasn't feces.

S. Muenster found predominant in this study (39.28%), was also the most prevalent serovar in each category of samples. According to Van Cauteren et al. (2009), this serovar is rarely identified from humans, foods or animals.

The other serovars recovered, including *S.* Kentucky, *S.* Infantis, *S.* Anatum, *S.* Typhimurium and *S.* Montevideo are, without respecting the ranked order, among the top 10 serovars encountered in Africa (Hendriksen et al., 2011), in Europe (EFSA, 2014), and in USA (CDC, 2014).

S. Kentucky was isolated from 15.47 % of samples. According to our results during this study and the international studies pre-established in Algeria (Bouzidi et al., 2012; Elgroud et al., 2015), Morocco (El Allaoui et al., 2014), Tunisia (Abbassi-Ghozzi et al., 2012), and Nigeria (Tafida et al., 2013), this result is alarming because the sudden emergence and worrying of *S.* Kentucky has shown an increasingly insensitive to almost all families of antibiotics.

Previous national studies demonstrated that *S.* Anatum was found to be the most prevalent serovar isolated from bovine and ovine carcasses (Nouichi and Hamdi, 2009) and from red meat and their products (Mezali and Hamdi, 2012) in Algiers. Otherwise, *S.* Typhimurium, *S.* Heidelberg, *S.* Enteritidis and *S.* Hadar were mainly isolated from broilers by Bounar-Kechih et al. (2012), Bouzidi et al. (2012), Elgroud et al. (2015), respectively. These findings suggest that several serovars of *Salmonella* that may cause significant animal and human illnesses occur in Algeria.

At the international level, *S.* Enteritidis and *S.* Typhimurium were reported to be the most frequently isolated (Hendriksen et al., 2011; CDC, 2014; EFSA, 2015).

These differences in the prevalence of *Salmonella* and the distribution of serovars between studies may depend on the isolation methodology, the geographic area, and the housing and husbandry conditions.

Molecular confirmation

The present study supports the ability of the S139 and S141 primers targeting the *inv*A gene specific of *Salmonella* spp. to confirm the isolated colonies.

Out of the 84 *Salmonella* isolates tested, 81 yield-

ed desired amplified products of approximately 284 bp similar to that of reference strain of *Salmonella* using the primer pairs for *inv*A. The efficiency was 96.43%. Our report corroborates many recent studies in Egypt (Maysa and Abd-Elall, 2015) and Nigeria (Smith et al., 2015) conducted on *Salmonella* isolated from human, animals, food and water samples in which *inv*A gene (284 bp) was prevalent at 96%. Karmi (2013) reported that all *Salmonella* isolates positive for the presence of *inv*A gene, have the capacity to invade and survive in macrophages.

In contrary, Karmi (2013), Tafida et al. (2013), and Dong et al. (2014) have detected and reported the *inv*A gene in all *Salmonella* isolates tested.

In this study, the DNA of three *Salmonella* isolates confirmed biochemically and serotyped (one *S.* Muenster, one *S.* Anatum, and one *S.* Infantis) was not amplified by PCR. Although, Rahn et al. (1992) could not detect S. Litchfield and S. Senftenberg by using S139 and S141 primers. Malorny et al. (2003) managed to do it with the same primers after modification in the thermal cycling conditions and using hot start PCR.

The failure to amplify *inv*A homologues sequences was most likely due to the absence of the *inv*A gene in these *Salmonella* strains (Rahn et al., 1992). Ginocchio et al. (1997) determined that the invasion-associated pathogenicity island which has previously been shown to be linked to the *inv* locus remains unstable in certain *Salmonella* serovars. It would also suggest that these organisms would not be invasive or, alternatively, that they may possess other pathways of invasion independent of *inv*A gene (Rahn et al., 1992; Ginocchio et al., 1997). In addition some *Salmonella* species also could not be detected by other PCR methods (Van Kessel et al., 2003).

Interestingly, the strains that were shown to lack of the *inv*A sequences in the two studies of Rahn et al. (1992) and Ginocchio et al. (1997) had been recovered from environmental samples and were not specifically associated with disease. Even whether the three isolates found negative for *inv*A gene during our study were isolated from carcasses; it is possible that all of them were originated from environment.

After identifying the *inv*A gene as specific to *Salmonella*, PCR has also been used for distinguishing of *Salmonella* serovars. As for *S.* Typhimurium, the *Salmonella* plasmid virulence s*pv* genes were used (Olsen et al., 2014). These genes are particularly required for systemic infection (Nickerson and Curtiss, 1997). In our study, all isolates serotyped *S.* Typhimurium were found positive for *spv* gene. The sensibility of the primer used in this study has also been also well demonstrated using simplex or multiplex PCR assays (Ahmed and Shimamoto, 2014; Can et al., 2014; Manoj et al., 2015).

Antimicrobial resistance of isolates strains

In the current study, the rate of resistance to at least one antimicrobial is high (80.95%) and could be explained by the widespread and indiscriminate use of the drugs for therapeutic and prophylactic purposes both in veterinary and human health sectors. This is in accordance with previous results recorded in Algeria (80% [Elgroud et al., 2009]; 68.42% [Bouzidi et al., 2012]; 90.32% [Mezali and Hamdi, 2012]), in Egypt (100% [Sallam et al., 2014]) and in Morocco (93.5% [El Allaoui et al., 2014]). Resistance to streptomycin was quite common (69.05%, n=58) and corroborates the finding of Elgroud et al. (2009; 58%) and Aouf et al. (2011; 68.75%), while 17.86% (n=15) of isolates were found resistant to tetracyclines, which is lower than that noted by Bouzidi et al. (2012; 36.9%) and Aouf et al. (2011; 100%). As for sulphonamides, our result (32.14%) was higher than that reported in Algeria by Bounar-Kechih et al. (2012) and Mezali and Hamdi (2012) who recorded 13% and 16.13%, respectively. Streptomycin, sulphonamides and tetracycline are old first-intention molecules and have been widely used in animal husbandry. In addition to streptomycin, resistance to aminoglycosides involved also gentamicin, (10.71%); all of the isolates resistant to this drug are belonging to *S.* Kentucky serovar. A similar result has been registered by Bouzidi et al. (2012) and Le Hello et al. (2013).

Only 2 isolates displayed resistance to the association trimethoprim/sulfamethoxazole, which is lower than the result of Aouf et al. (2011). In the present study, 15 (17.86%) isolates were resistant to nalidixic acid; 18.75% were recorded by Aouf et al. (2011). Increasing resistance to this antimicrobial has been

also reported by the national studies (Elgroud et al., 2009; Bounar-Kechih et al., 2012; Mezali and Hamdi, 2012).

As for furans and chloramphenicol 1.19% and 2.38% were recorded respectively. These findings corroborate those of Mezali and Hamdi (2012) and could be explained by the moderate use of these drugs because of their removing from the Algerian nomenclature.

Moreover, all of the *S.* Kentucky isolates were found to be resistant to fluoroquinolones (norfloxacin and/or ciprofloxacin). To our knowledge, this is the first national paper showing resistance to these drugs in *Salmonella* strains isolated from red meats since the other previous studies in Algeria (Elgroud et al., 2009; Bouzidi et al., 2012) were done on poultry.

This finding is more worrying as fluoroquinolones should be reserved for the treatment of serious gastrointestinal infections in adults. This may be linked to a non-prudent use of these molecules, although expensive in animal husbandries in Algeria (Elgroud et al., 2009).

Full resistance to quinolones is achieved when two cumulative mutations in genes that encode the targets of these drugs are present concurrently (Le Hello et al., 2013; El Allaoui et al., 2014).

It appears that isolates tested in this study underwent this kind of mutation only because of which they were resistant to nalidixic acid and to other quinolones molecules such as norfloxacin and ciprofloxacin. Resistance to β-lactams involved only penicillins (ampicillin, ticarcillin, piperacillin, amoxicillin, mecillinam and the combination amoxicillin/clavulanic acid) and first generation cephalosporins (cefazolin). Conversely, the absence of resistance to third generation cephalosporins was an important finding since they are clinically essential in the treatment of invasive salmonellosis in humans. Multiple drug resistance in *Salmonella* may result from random chromosomal mutations and transfer of resistance genes (Abbassi-Ghozzi et al., 2012). Multidrug resistant *Salmonella* serovars have been proposed to be more virulent than non-multidrug resistant ones (Sallam et al., 2014).

In our study, 14 different MDR patterns were found. The two serovars commonly involved in food-borne outbreaks, *S.* Kentucky and *S.* Typhimurium presented the greatest number of multi-resistance phenotypes. Transmission of multi-resistant *Salmonella* to humans through food chain may involve a high risk for public health by compromising the effectiveness of medical treatment and increasing the number of invasive infections. Two *S.* Typhimurium isolates displayed an ‘‘ACSSuT’’ pentaresistance pattern. This is another worrying antimicrobial pattern evidenced during this study was also found by Mezali and Hamdi in 2012. In Europe, resistance to ‘‘ACSSuT’’ was the most common multidrug-resistant pattern recorded among the multidrug-resistant *Salmonella* Typhimurium isolated from human and food sources (EFSA, 2015). All the strains displaying MDR were isolated from both ovine and bovine carcasses. By contrast, all *Salmonella* isolated from feces were resistant only to streptomycin or sulphonamides. This finding confirms that the high number of *Salmonella* isolated from carcasses during this study is not mainly associated with fecal carriage.

CONCLUSIONS

Overall, our findings showed that the bovine and ovine carcasses are considered as an important source of multidrug-resistant *Salmonella* serovars and can pose a high risk for the consumer; subsequently, hygienic measures should be undertaken to reduce contamination of meat with virulent strains of *Salmonella*, and strict guidelines for the use of antibiotics should be necessary to prevent the dissemination and acquisition of antimicrobial resistance. This study also demonstrated that 96.43% of *Salmonella* isolates were positive for the presence of virulence gene (*inv*A) that responsible for cell invasion. Furthermore, it is important to emphasize that PCR method based on *inv*A gene should be used for rapid identification of *Salmonella* serovars and could replace the conventional bacteriological and biochemical methods.

CONFLICT OF INTEREST STATEMENT

The authors report no conflict of interest.

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