Original Article

Antimicrobial and molecular analysis of *Salmonella* serovar Livingstone strains isolated from humans in Tunisia and Belgium

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Abstract

Introduction: *Salmonella* Livingstone is one of the most common serotypes responsible for nosocomial outbreaks in Tunisia. In this study, 42 isolates of *Salmonella* Livingstone were analyzed. Most of these were isolated from humans (31 strains from Tunisia and 9 strains from Belgium) and 2 isolates came from food products (beef and pork).

Methodology: All strains were characterized by antibiogram, multilocus sequence typing (MLST), and virulotyping. This last technique was carried out by simple PCR of five chromosomal genes (*agfA*, *hin/H2*, *iroB*, *phoP/Q*, and *slyA*) and two plasmid genes (*spvA* and *spvC*).

Results: All Tunisian strains were resistant to amoxicillin, amoxicillin-clavulanic acid, ticarcillin, cefalotin, gentamicin, and kanamycin. They were also resistant to third-generation cephalosporin antibiotics (cefotaxim and ceftazidim). Belgian isolates were susceptible to all antibiotics tested. Further to MLST analyses, Tunisian strains belonged to the same sequence type, ST543. For Belgian isolates, eight strains had a ST543 profile, two strains had a ST638 profile, and one strain had a ST457 profile. Analyses of the virulence gene contents showed that strains isolated in different years and from different origins had the same virulence profile. These carried all five chromosomal genes and lacked plasmid-located virulence genes *spvA* and *spv*C.

Conclusions: A combination of different typing methods showed that the majority of Belgian strains and all Tunisian strains were closely related; they belonged to the same sequence type (ST543) and had the same virulence profile, but different antibiotic resistance profiles depended on the country of origin.

Key words: *Salmonella* Livingstone; MLST; virulence genes; antimicrobial resistance.

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Introduction

Salmonella enterica is among the most important causes of foodborne gastroenteritis worldwide [1-2]. It is also responsible for nosocomial outbreaks, particularly in developing countries. *Salmonella enterica* serovar Livingstone is one of the more than 2,500 serovars of *S. enterica* known [3]. It was first isolated from human feces in 1951 [4]; since then, it has been rarely isolated from human beings and animals, particularly poultry and feed products [5-6].

In Tunisia, there was a dramatic increase in the number of isolations of *Salmonella* Livingstone from humans and animals between 1999 and 2003 [7]. This particular serotype was one of the most common *Salmonell*a isolates responsible for nosocomial outbreaks in Tunisian hospitals, especially in pediatric wards [8-9].

In Europe, the number of reported sporadic human cases of *Salmonella* Livingstone infections was low, and outbreaks were rare [10]. The larger outbreaks were recorded in Tayside, Scotland, during 1989–1991 [5], and in several European countries in 1996 related to travel to Tunisia [11]. In Belgium, *Salmonella* Enteritidis and *S.* Typhimurium were the most common serovars causing salmonellosis in humans; *Salmonella* Livingstone was rarely isolated [12].

Numerous phenotypic and genotypic methods, among them biotyping, antimicrobial susceptibility profiling, plasmid profiling, ribotyping, random amplification of polymorphic DNA (RAPD-PCR), enterobacterial repetitive intergenic consensus (ERIC-PCR) and pulsed field gel electrophoresis (PFGE) have been used to subtype *Salmonella* serotype Livingstone and to investigate outbreaks caused by

this serotype [7,9,13]. Currently, alternative molecular typing methods such as multilocus sequence typing (MLST) and virulotyping [1,14-15] are used to characterize many *Salmonella enterica* serovars. Multilocus sequence typing [16] has been increasingly recognized as a method of choice for genotyping a number of bacterial pathogens [17-18], including *Salmonella* [1,19-21], and has been used successfully in epidemiological studies and outbreak investigations [17,22]. This technique is based on determination of the DNA sequence of a series of selected housekeeping, ribosomal, and/or virulence-associated genes [23-24].

Virulence genes have been screened using simple [25] or multiplex PCR [26] to determine the distribution of these genes among *Salmonella enterica* strains [25,27] and to genotype many *Salmonella* serovars [15,28-29].

The objective of this study was to investigate the phylogenetic relationship between clinical *Salmonella* Livingstone strains isolated from Tunisia and Belgium between 2002 and 2010 and to analyze the differences in antimicrobial susceptibility profiles and the genomic content of some well-known determinants of virulence in these isolates.

Methodology

Bacterial strains

Forty-two strains of *Salmonella* Livingstone were included in this study, of which 31 were collected from human stools between 2002 and 2009 in Farhat Hached University Hospital located in the town of Sousse (central-east of Tunisia).

The majority of these strains (n = 27) were recovered from stool specimens of babies up to two months of age, during a nosocomial outbreak that occurred in the neonatology ward of the maternity department in 2005. Nine unrelated strains isolated in 2010 from humans were provided by Belgium's National Reference Centre for *Salmonella* and *Shigella*. Two Belgian meat isolates (pork and beef) were used as comparison strains (Table 1).

All isolates were identified with the API 20E system (bio-Mérieux, Marcy l'Etoile, France) and were serotyped by agglutination tests with antisera (Bio-Rad, Hercules, USA) as specified by the White-Kauffmann-Le Minor scheme [30].

Antimicrobial susceptility testing

Antibiotic susceptibility was determined by the disk diffusion method on Mueller-Hinton agar (Bio-Rad) according to the guidelines of the Antibiogram Committee of the French Society for Microbiology (CA-SFM).

The following antimicrobial agents (Bio-Rad) were tested: amoxicillin (AMX, 25 µg), amoxicillin-clavulanic acid (AMC, 20/10 µg), ticarcillin (TIC, 75 µg), ticarcillin-clavulanic acid (TCC, 75/10 µg), piperacillin (PIP, 75 µg), imipenem (IMP, 10 µg), cefalotin (CF, 30 µg), cefoxitin (FOX, 30 µg), cefotaxim (CTX, 30 µg), ceftazidime (CAZ, 30 µg), kanamycin (K, 30 UI), tobramycin (TB, 10 µg), gentamycin (GM, 15 µg), amikacin (AN, 30 µg), nalidixic acid (NA, 30 µg), ofloxacin (OFX, 5 µg), ciprofloxacin (CIP, 5 µg), colistin (CS, 50 µg), trimethoprim (TM, 5 µg), chloramphenicol (C, 30 µg), fosfomycin (FOS, 50 µg), tetracyclin (TE, 30 UI), and trimethoprim sulfathomexazole (SXT, 1.25/23.75 µg).

Multilocus sequence typing (MLST)

Multilocus sequence type analysis was carried out using the MLST protocols described on the MLST website (MLST.net).

Briefly, all isolates were grown overnight in LB medium at 37°C and total DNA was extracted by thermal extraction. For each strain, two to three colonies were dissociated in 150 µL of sterile distilled water; this suspension was heated to 95°C for five minutes, then cooled immediately in ice. After two minutes of centrifugation at 13,000 rpm, the supernatant was conserved at -20°C [31].

PCR amplification for the seven genes of the MLST panel was carried out as follows: primer pairs (Table 2) were used to amplify the DNA for the presence of the following genes: *aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA*, and *thrA*. PCR reactions were carried out in 20 µL volumes containing 2 µL of DNA template, 5 U/µL of Taq DNA polymerase (Eurogentec, Seraing, Belgium), 1X PCR buffer, 0.5 µM of forward and reverse primers (Eurogentec, Seraing, Belgium), and 200 µM of DNTPs (Promega Fitchburg, USA).

PCR cycling conditions were 94°C for 5 minutes followed by 34 cycles of 94°C for 1 minute, 55°C for 1 minute and 72°C for 1 minute, with a final extension of 72°C for 5 minutes followed by hold at 4°C.

Table 1. Origin and characterization of *Salmonella* Livingstone isolates used in this study

Isolate	Source	Country	Year	Resistance profile	ST type
TN1	Human, stools	Tunisia	2002	AMX-AMC-TIC-TCC-PIP-CF-CTX-CAZ-K-G-Tb-TM-SXT	ST543
TN2	Human, stools	Tunisia	2004	AMX-AMC-TIC-TCC-PIP-CF-CTX-CAZ-K-G-Tb-TM-SXT	ST543
TN3	Human, stools	Tunisia	2005	AMX-AMC-TIC-TCC-PIP-CF-CTX-CAZ-K-G-Tb-TM-SXT	ST543
TN4	Human, stools	Tunisia	2005	AMX-AMC-TIC-TCC-PIP-CF-CTX-CAZ-K-G-Tb-TM-SXT	ST543
TN5	Human, stools	Tunisia	2005	AMX-AMC-TIC-TCC-PIP-CF-CTX-CAZ-K-G-Tb-TM-SXT	ST543
TN6	Human, stools	Tunisia	2005	AMX-AMC-TIC-TCC-PIP-CF-CTX-CAZ-K-G-Tb-TM-SXT	ST543
TN7	Human, stools	Tunisia	2005	AMX-AMC-TIC-TCC-PIP-CF-CTX-CAZ-K-G-Tb-TM-SXT	ST543
TN8	Human, stools	Tunisia	2005	AMX-AMC-TIC-TCC-PIP-CF-CTX-CAZ-K-G-Tb-TM-SXT	ST543
TN9	Human, stools	Tunisia	2005	AMX-AMC-TIC-TCC-PIP-CF-CTX-CAZ-K-G-Tb-TM-SXT	ST543
TN10	Human, stools	Tunisia	2005	AMX-AMC-TIC-TCC-PIP-CF-CTX-CAZ-K-G-Tb-TM-SXT	ST543
TN11	Human, stools	Tunisia	2005	AMX-AMC-TIC-TCC-PIP-CF-CTX-CAZ-K-G-Tb-TM-SXT	ST543
TN12	Human, stools	Tunisia	2005	AMX-AMC-TIC-TCC-PIP-CF-CTX-CAZ-K-G-Tb-TM-SXT	ST543
TN13	Human, stools	Tunisia	2005	AMX-AMC-TIC-TCC-PIP-CF-CTX-CAZ-K-G-Tb-TM-SXT	ST543
TN14	Human, stools	Tunisia	2005	AMX-AMC-TIC-TCC-PIP-CF-CTX-CAZ-K-G-Tb-TM-SXT	ST543
TN15	Human, stools	Tunisia	2005	AMX-AMC-TIC-TCC-PIP-CF-CTX-CAZ-K-G-Tb-TM-SXT	ST543
TN16	Human, stools	Tunisia	2005	AMX-AMC-TIC-TCC-PIP-CF-CTX-CAZ-K-G-Tb-TM-SXT	ST543
TN17	Human, stools	Tunisia	2005	AMX-AMC-TIC-TCC-PIP-CF-CTX-CAZ-K-G-Tb-TM-SXT	ST543
TN18	Human, stools	Tunisia	2005	AMX-AMC-TIC-TCC-PIP-CF-CTX-CAZ-K-G-Tb-TM-SXT	ST543
TN19	Human, stools	Tunisia	2005	AMX-AMC-TIC-TCC-PIP-CF-CTX-CAZ-K-G-Tb-AN-TM-SXT	ST543
TN20	Human, stools	Tunisia	2005	AMX-AMC-TIC-TCC-PIP-CF-CTX-CAZ-K-G-Tb-TM-SXT	ST543
TN21	Human, stools	Tunisia	2005	AMX-AMC-TIC-TCC-PIP-CF-CTX-CAZ-K-G-Tb-TM-SXT	ST543
TN22	Human, stools	Tunisia	2005	AMX-AMC-TIC-TCC-PIP-CF-CTX-CAZ-K-G-Tb-TM-SXT	ST543
TN23	Human, stools	Tunisia	2005	AMX-AMC-TIC-TCC-PIP-CF-CTX-CAZ-K-G-Tb-TM-SXT	ST543
TN24	Human, stools	Tunisia	2005	AMX-AMC-TIC-TCC-PIP-CF-CTX-CAZ-K-G-Tb-TM-SXT	ST543
TN25	Human, stools	Tunisia	2005	AMX-AMC-TIC-TCC-PIP-CF-CTX-CAZ-K-G-Tb-TM-SXT-TE	ST543
TN26	Human, stools	Tunisia	2005	AMX-AMC-TIC-TCC-PIP-CF-CTX-CAZ-K-G-Tb-FOX(I)-TM-SXT-TE	ST543
TN27	Human, stools	Tunisia	2005	AMX-AMC-TIC-TCC-PIP-CF-CTX-CAZ-K-G-Tb-TM-SXT	ST543
TN28	Human, stools	Tunisia	2005	AMX-AMC-TIC-TCC-PIP-CF-CTX-CAZ-K-G-Tb-FOX(I)-TM-SXT-TE	ST543
TN29	Human, stools	Tunisia	2005	AMX-AMC-TIC-TCC-PIP-CF-CTX-CAZ-K-G-Tb-AN-FOS-TM-SXT-TE	ST543
TN30	Human, stools	Tunisia	2009	AMX-AMC-TIC-TCC-PIP-CF-CTX-CAZ-K-G-Tb-TM-SXT	ST543
TN31	Human, stools	Tunisia	2009	AMX-AMC-TIC-TCC-PIP-CF-CTX-CAZ-K-G-Tb-FOX(I)-TM-SXT	ST543
BE1	Human	SNRC, Belgium	2010	Susceptible	ST638
BE2	Human	SNRC, Belgium	2010	Susceptible	ST543
BE3	Human	SNRC, Belgium	2010	Susceptible	ST457
BE4	Human	SNRC, Belgium	2010	Susceptible	ST543
BE5	Human	SNRC, Belgium	2010	Susceptible	ST638
BE6	Human	SNRC, Belgium	2010	Susceptible	ST543
BE7	Human	SNRC, Belgium	2010	Susceptible	ST543
BE8	Human	SNRC, Belgium	2010	Susceptible	ST543
BE9	Human	SNRC, Belgium	2010	Susceptible	ST543
BE11	beaf,meat	FSD[c], Belgium	2004	Susceptible	ST543
BE12	porc, meat	FSD, Belgium	2006	Susceptible	ST543

PCR products were purified with a PCR purification kit from Promega, and two independent amplified fragments were sequenced using the primers SE-Univfor and SE-Univrev (Table 2).

Sequencing was done with the Sanger sequencing (Big Dye) method and an ABI 3730 capillary sequencer (http://www.giga.ulg.ac.be/jcms/c_5015/fr/accueil).

The sequences were submitted to the MLST database website (http://mlst.ucc.ie/mlst/dbs/Senterica) and assigned existing or novel allele type numbers and sequence type numbers defined by the database. This database defined a novel allele type if it contained one or more nucleotide changes from existing allele sequences. Composite sequence types (STs) were assigned based on the set of allele types derived from each of the seven loci.

Virulotype PCR screening

The PCR-based assays were described by Del Cerro *et al*. [32]. Seven *Salmonella* virulence genes were screened in all the isolates, including five chromosomally located genes (*agfA*, *hin/H2*, *iroB*, *phoP/Q* and *slyA*) and two plasmid-encoded virulence factors (*spvA* and *spvC*) (Table 3). These virulence determinants represented regions known to be either

Table 2. PCR and sequencing primers used for MLST

Gene	Primer designation	Sequence (5'-3')	Size of PCR product (bp)
aroC	SE-aroC for	GTTTTCCCAGTCACGACGTTGTACCTGGCACCTCGCGCTATAC	826
	SE-aroC rev	TTGTGAGCGGATAACAATTTCCCACACACGGATCGTGGCG	
dnaN	SE-dnaN for	GTTTTCCCAGTCACGACGTTGTAATGAAATTTACCGTTGAACGTGA	833
	SE-dnaN rev	TTGTGAGCGGATAACAATTTCAATTTCTCATTCGAGAGGATTGC	
hemD	SE-hemD for	GTTTTCCCAGTCACGACGTTGTAGAAGCGTTAGTGAGCCGTCTGCG	666
	SE-hemD rev	TTGTGAGCGGATAACAATTTCATCAGCGACCTTAATATCTTGCCA	
hisD	SE-hisD for	GTTTTCCCAGTCACGACGTTGTAGAAACGTTCCATTCCGCGCAGAC	894
	SE-hisD rev	TTGTGAGCGGATAACAATTTCCTGAACGGTCATCCGTTTCTG	
purE	SE-purE for	GTTTTCCCAGTCACGACGTTGTAATGTCTTCCCGCAATAATCC	510
	SE-purE rev	TTGTGAGCGGATAACAATTTCTCATAGCGTCCCCCGCGGATC	
sucA	SE-sucA for	GTTTTCCCAGTCACGACGTTGTAAGCACCGAAGAGAAACGCTG	643
	SE-sucA rev	TTGTGAGCGGATAACAATTTCGGTTGTTGATAACGATACGTAC	
thrA	SE-thrA for	GTTTTCCCAGTCACGACGTTGTAGTCACGGTGATCGATCCGGT	852
	SE-thrA rev	TTGTGAGCGGATAACAATTTCCACGATATTGATATTAGCCCG	
		Sequencing Primers	
SE-Univ	SE-Univfor	GTTTTCCCAGTCACGACGTTGTA	
	SE-Univrev	TTGTGAGCGGATAACAATTTC	

[a]SNRC: *Salmonella* National Reference Center; [b] FSD*:* Food Sciences Department

Table 3. Target genes for virulo-polymerase chain reaction screening

Target		Oligonucleotide primers
		Sequence (5'–3')
iroB gene	Fw	TGCGTATTCTGTTTGTCGGTCC
	Rev	TACGTTCCCACCATTCTTCCC
agfA gene	Fw	TCCGGCCCGGACTCAACG
	Rev	CAGCGCGGCGTTATACCG
*hin/H2 g*ene	Fw	CTAGTGAAATTGTGACCGCA
	Rev	CCCATCGCGCTACTGGTATC
phoP/Q gene	Fw	ATGCAAAGCCCGACCATGACG
	Rev	GTATCGACCACCACGATGGTT
slyA gene	Fw	GCCAAAACTGAAGCTACAGGTG
	Rev	CGGCAGGTCAGCGTGTCGTGC
spvA gene	Fw	GTCAGACCCGTAAACAGT
	Rev	GCACGCAGAGTACCCGCA
spvC gene	Fw	ACTCCTTGCACAACCAAATGCGGA
	Rev	TGTCTTCTGCATTTCGCCACCATCA

highly conserved (SPIs) or variable (plasmid) within the *Salmonella* genome.

The program consisted of a hot start cycle 94°C for 5 minutes, followed by 30 cycles of 94°C for 30 seconds, 60°C for 30 seconds and 72°C for 1 minute, and a final extension step of 72°C for 5 minutes. In all experiments, a negative control without template DNA was introduced. Aliquots of 10 µL of amplification products were analyzed by electrophoresis on 1.5% agarose gels and run in TAE buffer.

Results

Antibiotic susceptibility

Susceptibility test by the disk diffusion method demonstrated that all Tunisian isolates (n = 31) were resistant to amoxicillin, amoxicillin-clavulanic acid, ticarcillin, ticarcillin-clavulanic acid, piperacillin, cefalotin, ceftazidim, cefotaxim, trimathoprim, and trimathoprim sulfathomexazol. These isolates were also resistant to aminoglygosides (kanamycin, gentamicin, and tobramycin). Only two isolates were resistant to amikacin. None of the Tunisian isolates were resistant to nalidixic acid, ofloxacin, chloromphenicol, colistin, ciprofloxacin, or imipinem. Four isolates were resistant to tetracycline, and three strains had intermediate resistance to cefoxitin. Belgian isolates were susceptible to all antibiotics tested (Table 1).

MLST

MLST consists of the sequencing of seven housekeeping genes dispersed over the entire genome. A *Salmonella* Typhi isolate was used as an outgroup. Overall, in the seven genes, MLST defined three sequence types: ST543 (n = 39), ST638 (n = 2), and ST457 (n = 1). The 31 *Salmonella* Livingstone isolated in the neonatology ward (Hospital of Sousse, Tunisia) between 2002 and 2009 were related. They possessed identical alleles at seven loci and were all assigned to ST543. The nine clinical isolates recovered from humans in Belgium belonged to three different sequence types: ST543 (n = 6), ST638 (n = 2), and ST457 (n = 1). The strains isolated from pork and beef meat in 2004 and 2008 had the same allelic profile and belonged to the ST543 sequence type. ST543 was the most commonly detected sequence type among Tunisian and Belgian isolates.

Virulotyping

The presence or absence of seven selected virulence determinants in the panel of 42 *Salmonella enterica* serotype Livingstone strains, including 40 strains isolated from humans and two strains from food products, was investigated by PCR.

All *Salmonella* isolates tested had the same virulence profile. They carried all tested chromosomal virulence genes: *agfA* (thin aggregative fimbriae), *hin/H2* (flagellar phase variation), *iroB* (regulation by iron), *phoP/Q* (intra-macrophage survival and enhanced bile resistance), and *slyA* (salmolysin), and lacked *spvA* and *spvC* genes, which were plasmid-located virulence genes.

Discussion

The purpose of this study was to investigate phenotypic and genotypic diversity among clinical strains of *Salmonella* Livingstone isolates from Tunisia and Belgium between 2002 and 2010. These isolates were subtyped by three well-established typing methods that had been shown to provide adequate discriminatory power for other serotypes.

MLST is becoming an evolutionary method for typing many bacterial pathogens [33-34]. Many MLST sequence typing schemes have been described for a variety of different *Salmonella* serotypes [1,14,35-36], but only limited information is available on the subtype by MLST of *Salmonella* Livingstone [21,37]. In the present report, we characterized a set a 42 *Salmonella* Livingstone isolates from Tunisia and Belgium by MLST based on sequencing of seven housekeeping genes (genes required for basic cellular functions).

Previous studies showed that MLST of several housekeeping genes may be a good molecular epidemiological option for discriminating among isolates that were shown to be indistinguishable by PFGE [38-39]. In our study, we found that the majority of *Salmonella* Livingstone tested by MLST (39/42) had the same allelic profile without any nucleotide differences in the seven genes and belonged to the ST543 sequence type. This finding was in agreement with earlier studies, which showed that isolates with the same serotype often had a similar sequence type, and that in general, STs were restricted to the same serovar [40-41].

Recently, many authors proposed new MLST schemes based on sequencing of other genes with higher discriminatory ability than housekeeping genes. They deduced that MLST using the combination of housekeeping genes and virulence or flagellin genes was more discriminatory than MLST using only housekeeping genes [21,38,42-43].

Persistence of the same clone of *S.* Livingstone in the hospital of Sousse over many years supports the

data that ST543 lineage had possibly acquired a niche in the neonatology ward since the first nosocomial outbreak occurred in 2002. In previous studies [8,13], genetic analysis by PFGE of serotype Livingstone isolates collected from different hospitals in Tunisia showed that a single clone of *Salmonella enterica* serotype Livingstone was responsible for an inter-hospital outbreak in the pediatric ward of Sfax hospital (South of Tunisia) and also the outbreaks in Monastir (central-east) and Tunis (north) hospitals. It seems that the ST543 clone identified in our study may be the same clone that emerged in different hospitals in Tunisia. MLST analysis of *Salmonella enterica* serotype Livingstone strains isolated from humans (n = 9) and food products (n = 2) in Belgium by MLST showed a genetic diversity among these isolates. In fact, three different sequence types were identified: ST543 (n = 8), ST638 (n = 2), and ST457 (n = 1). Strains isolated from pork and beef had the same allelic profile as the majority of clinical strains isolated in Tunisia and Belgium and belonged to the ST543 sequence type. The same sequence type has also been detected in food samples in Thailand [44]. More isolates from different parts of the world and from different origins need to be tested to confirm that the ST543 sequence type was a major worldwide clone based on MLST analysis. Several PCR-based procedures for the detection of known DNA sequences, including virulence genes, have been successfully applied for many *Salmonella* serovars [15,25,28-29,45-46]. The main advantages of these approaches were the simplicity, rapidity, and cost effectiveness, which means that many strains could be easily virulotyped [15,32] . In this study, we used the same primers of virulence genes developed previously by Del Cerro *et al*. [32] to characterize *Salmonella* strains isolated from humans and from animals. We found that all *Salmonella* Livingstone analyzed had the same virulence gene profile (virulotype). They carried all the chromosomal genes tested (*phoP/Q*, *iroB*, *slyA*, *hin*/*H2*, and *agfA*) and lacked the plasmid virulence genes *spvA* and *spvC*. The same profile was observed for *S.* Panama and *S.* Wien, whereas *S.* Enteritidis and *S.* Derby lacked the *hin/H2* gene. *Salmonella* Hadar and *Salmonella* Typhimirium usually lacked the *agfA* gene [32]. Heun *et al*. [15] suggested that the virulotype did not vary with the host source or geographical location. Our results confirmed this data. In fact, the strains of *Salmonella* Livingstone isolated from food products and from humans in two different countries had the same virulence profile. Another study conducted by Soto *et al*. [28] showed that 86 *Salmonella* Panama strains isolated from different origins (humans, octopi, beef, eggs, sea water, and sewage) had the same virulence profile. On the other hand, the *spv* operon is present in large plasmids found in few serotypes of *Salmonella* subspecies I [47-48]. This locus harbors five genes designated *spvRABCD*. Expression of the *spv* genes might play an important role in systemic infection and survival of *Salmonella* in the reticulo-endothelial system [48]. The absence of plasmid virulence genes for all *Salmonella* Livingstone strains tested in this study was an indicator of low virulence of these strains. This result confirmed the hypothesis of Reilly *et al*. [6], suggesting that this *Salmonella* serotype might have low virulence for humans. The absence of *spv* genes may be explained by the origin of the isolates tested. In fact, all clinical *Salmonella* Livingstone strains were isolated from patients suffering from gastroenteritis. It has been proven that the *spv* locus is strongly associated with strains that cause non-typhoid bacteremia [49].

Heitchoff *et al*. [27] reported that *Salmonella* Typhimurium derived from gastroenteritis patients did not possess the *Salmonella* virulence plasmid, in contrast to all animal and human bacteremia isolates tested.

In Tunisia, there was a significant increase in the number of the non-typhoid *Salmonella* isolates expressing resistance to extended spectrum cephalosporins (ESC). It has been reported that these isolates were the major cause of nosocomial outbreaks in pediatric wards in Tunisia, and involved various serotypes, including Wien, Mabandaka, and Livingstone [9]. *Salmonella* Livingstone producing CTX-M-27 and ACC1 has been reported in Tunisia [7-8]. In this report, *Salmonella* Livingstone strains collected during the outbreak occurred in the neonatology ward in 2005 and strains isolated in individual cases in 2002, 2004, and 2009 were all resistant to the majority of antibiotics tested and had a particular resistance to third-generation cephalosporins (cefotaxim and ceftazidim). To confirm that these strains were producers of β-lactamases, it is important to conduct further phenotypic tests (diffusion test, CMI) followed by genetic analysis (amplification, sequencing). On the other hand, all isolates collected in Belgium from humans and food products were susceptible to all antibiotics tested.

Conclusions

The combination of results of the three typing methods showed no correlation between multilocus

sequence typing, virulotyping, and antibiotic profiling. In fact, we found that strains that belonged to the same virulotype can have different antibiotic profiles and belong to different genotypes.

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