Original Article

Isolation, drug resistance and molecular characterization of *Salmonella* isolates in northern Morocco

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Abstract

Objectives: The objective of this investigation was to assess the methods for the characterization of *Salmonella* isolates and to identify relationships of *Salmonella* isolates from human and food sources in northern Morocco.

Methodology: Several *Salmonella* serotypes were isolated from human and food samples and were characterized using conventional culture methods, biochemical, serological, antimicrobial testing, and phage typing. Molecular analyses such as enterobacterial repetitive intergenic consensus (ERIC)-PCR, macrorestriction profiling by pulsed-field gel electrophoresis (PFGE), and virulence gene analysis were also performed.

Results: Sixteen *Salmonella* strains were isolated in our laboratory, serotyped and identified as *S.* Kottbus, *S.* Indiana, *S.* London, *S.* Typhi, *S.* Hadar, *S.* Corvallis, *S.* Mbandaka, *S.* Ouakam, *S.* Tm var. cop., *S.* Virchow, and *S.* Altona. The most common resistance profiles for the isolates was ATCFATSCGKSSS, belonging to phage type PT20, ATASCSS associated with strains DT104L/ad and ATATSS for isolates that were not typeable. The PFGE patterns were different for each *Salmonella* serotype. All strains were negative for the virulence gene *spv*R.

Conclusions: To our knowledge, this is the first molecular characterization of *Salmonella* in food and humans from Morocco. Comparison of molecular techniques for differentiating between human and food isolates of *Salmonella* in north of Morocco shows that ERIC typing and PFGE were more discriminating than the other techniques used in this study.

Key Words: Epidemiology; *Salmonella*; PFGE; antimicrobial resistance; phage typing

J Infect Developing Countries 2009; 3(1):41-49.

Received 16 July 2008 – Accepted 20 October 2008

Introduction

Salmonella infections occur worldwide in both developed and developing countries and are a major contributor to morbidity and economic costs [1]. The contamination of food products with *Salmonella* generates serious health and economic consequences, which have stimulated numerous studies designed to investigate the survival capacity and the transmission routes of these organisms in different farm animals and the environment [2,3]. *Salmonella* is frequently isolated from environmental sources that serve as a relay for the bacteria and play a major role in its spread between different hosts [4].

Salmonella can colonize and cause disease in a variety of food-producing and non-food-producing animals. Within this genus, more than 2,500 serovars have been described [5,6]. Although all serovars may be regarded as potential human pathogens, the majority of infections are caused by a very limited number of serovars. Since these pathogens are transmitted primarily through contaminated food or water, the presence of strains in food animals and ultimately in raw meat products has important public health implications [6,7].

All over the world, the serotype most often isolated is *S.* Enteritidis [1]. Recent reports from England and Spain show *S.* Enteritidis as the most frequent serotype with incidences of 60% and 86%, respectively [8]. Between 2000 and 2002, *S.* Enteritidis and *S.* Typhimurium represented 53% and 20 %, respectively, of the strains (n = 232 442) reported to the Enter-net in Europe [8]. In Casablanca, Morocco, the most prevalent serotypes are *S.* Typhimurium and *S.* Enteritidis [9].

To investigate the origins and the relationships among different isolates, more precise methods than serotyping are needed. Methods used for the subtyping of *Salmonella* include antibiotic susceptibility, pulsed-field gel electrophoresis typing (PFGE), phage typing, plasmid profiling, ribotyping, and randomly amplified polymorphic DNA analysis [1].

The molecular typing methods such as the REP-PCR [10-11] and RAPD-PCR methods [12] can be used for the differentiation and characterization of *Salmonella* and to trace the clonality of strains [13]. These repetitive elements include the repetitive extragenic palindronic (REP) elements [14] and the enterobacterial repetitive intergenic consensus (ERIC) sequences [15]. These primers (ERIC) were used to differentiate between closely related bacterial strains in several studies [16,17,18].

Among molecular techniques, PFGE is currently considered one of the most reliable typing procedures. This method is a well established and highly effective epidemiological tool for the molecular analysis of large fragments generated by restriction endonuclease digestion of genomic DNA. Very recently, the use of PFGE with endonuclease *Xba*I has been widely recognized as a sensitive means of fingerprinting *Salmonella* serovars and it has become a reference method [19,20,21].

During salmonellosis, the invasion of *Salmonella* into the small intestine is attributed to a number of invasion genes; one of these genes is *inv*A. This gene has been the target sequence for a number of rapid detection methods for *Salmonella* in foods. Another set of virulence genes includes the plasmid-encoded *spvABCD* and R genes found in some non-typhoidal *Salmonella* strains [22].

In the present study, we analyzed strains of *Salmonella* isolated from human stools and from food in the north of Morocco. This study was conducted to identify and establish the antimicrobial susceptibility pattern of the *Salmonella* and to determine the lysotype of these *Salmonella*. We use enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR), plasmid profile and PFGE as molecular techniques to distinguish between the isolates. Furthermore, the detection of the virulent genes such as *spvR* and *invA* was also performed. To our knowledge, this represents the first study of this type in the north of Morocco

Thus, the aim of this study was to investigate the diversity of *Salmonella* isolates from human samples and food in north of Morocco, to compare phenotyping and genotyping methods for differentiating *Salmonella* isolates, and to compare the different serotype of *Salmonella*. The study further aimed to determine the phenotypic and genotypic characteristics, including the antibiotic resistance, phage typing, plasmid profiles, PFGE, and the presence of virulence genes *inv*A and *spv*R, of the isolates.

Materials and Methods

Salmonella sources and isolation

The human *Salmonella* isolates were obtained from 100 acute diarrhoeal stool cultures of patients directed to Pasteur Laboratories from five medical centers and the regional hospital involved in this study. The samples were collected between February 2005 and August 2006. All samples were analysed for isolation of *Salmonella* strains using the French AFNOR method V [23]. The specimens transported in Cary-Blair media were plated directly onto Gelatin Agar, and were inoculated into Selenite broth and on *Salmonella–Shigella* (SS) and Hektoen Agars. Hektoen and SS were incubated at 37°C for 18 to 24 hours, and Selenite broth at 37°C for 12 to 18 hours. After incubation, Selenite broth was inoculated in SS agar and Hektoen at 37°C for 18 to 24 hours.

Food specimens were received at the *Service Agroalimentaire* of the Pasteur Institute and were comprised of chicken meat and other foods such as eggs and visceral organs collected from many markets in the region of Tangier at the same period of time as the feces collection. A total of 432 samples were aseptically collected on a random basis from different localities (different markets) in northern Morocco.

Approximately 25 g of food were placed in 225 ml of Buffered Peptone Water (BPW) as pre-enrichment media, and incubated at 37°C for 18 hours. After incubation, 0.1ml of the BPW was added to Rappaport–Varsiliadis broth, an enrichment media, and incubated at 42°C for 18 hours. A swab of the broth was inoculated onto Hektoen selective media.

Suspected colonies for *Salmonella* were inoculated in Urea Indol at 37°C for 2 to 4 hours, in Hajna Kligler at 37°C for 18 to 24 hours, and with an ONPG disc for biochemical testing and presumptive identification.

All isolates were biochemically identified by using the API20-E system (bioMérieux SA, Marcy–l'Étoile France). All strains were stored frozen at -

80°C in 20% Glycerol and in conservation Agar cultures at room temperature.

Serotyping and phage typing

Serotyping of *Salmonella* isolates was performed by slide agglutination with commercial antisera following the Kauffmann-White serotyping scheme of Popof and Le Minor [24], in collaboration with the National Reference center for *Salmonella* and *Shigella*, Brussels, Belgium.

Phage typing was performed according to the recommendations of the Health Protection Agency Service (Colindale, UK) (16) in the Pasteur Institute, Brussels, Belgium. The phage tying was conducted for only three serotypes of *Salmonella*: *S.* Hadar, *S.* Typhi and *S.* Virchow.

Antimicrobial drug resistance

Antibiotic susceptibility testing was performed by a disc diffusion method on Mueller-Hinton agar and interpreted in accordance with the criteria of the National Committee for Clinical Laboratory Standards [25]. The strains were screened for their resistance to the following antibiotics (Sanofi Diagnostics Pasteur): amoxicillin, AMX 25 µg; furan, F 30 µg; ticarcillin, TIC 75 µg; cephalothin, CEF 30 µg; gentamicin, GEN 15 µg; tobramycin, TOB 10 µg; nalidixicacid, NAL 30 µg; norfloxacin, NOR 5 µg; ciprofloxacin, CIP 5µg; ceftazidime, CAZ 30 µg; amoxicillin-clavulanic acid, AMC 20+10 µg; amikacin, AMK 30 µg; cefoxitin, FOX 30 µg; cefotaxime, CTX 30 µg; ampicillin, AMP 10µg. We used the Automated System (OSIRIS) for reading and interpreting results (Bio-Rad).

Macrorestriction analysis (PFGE)

Genomic DNA suitable for pulsed-field gel electrophoresis (PFGE) was prepared according to the PulseNet method (www.cdc.gov/pulsenet) and digested with the restriction endonuclease *Xba*I (New-England Biolab, Leudsen, Netherlands). *Salmonella* Braenderup H9812 was used as a size marker. Fingerprinting II (Informatix™ Software) (Bio-Rad) was used to compare the PFGE profiles. The bands generated were analyzed by using the Dice coefficient and the unweighted pair group method with averages (UPGMA) using a tolerance of (1%).

ERIC-PCR analysis

For enterobacterial repetitive intergenic consensus (ERIC) PCR, total *Salmonella* DNA was extracted using the Insta Gene Matrix Kit **(**Bio-Rad, Marnes-la-Coquette, France). ERIC-PCR was performed as previously described by BEYER *et al.* [26], using oligonucleotide sequences corresponding to the *eric* gene [ERIC1 (5'-ATG TAA GCT CCT GGG GAT TCA C-3') and ERIC2 (5'-AAG TAA GTACTGGGGTGAGCG-3')]. The PCR products were separated in 1.5% agarose gel containing ethidium bromide. The size of the PCR product was determined by comparison to the 100-bp DNA ladder (EZ Load 100 bp, Bio-Rad Laboratories) as a molecular marker, photographed and analyzed under UV light in gel-doc system (BioRad, MuK nchen, Germany).

PCR for spv and inv regions and plasmid preparation

PCR for the *spv* region was performed as described by Rubino *et al.* [27], using oligonucleotide sequences corresponding to the *spv*R gene of the *Salmonella* virulence plasmid (*spv*R3 5'–CCCCGGGAATTCGCTGCAT AAGGTCAGAAGG-3' and *spv*R5 5'-CCCCGGGATCCATGGATTTCTTGATTAATAA A-3') that amplify a fragment of 890 bp. Amplification of the *inv* region was performed as described by Gregory *et al.* [29] by using oligonucleotide sequences corresponding to the *inv* gene (invasion gene) *inv*A1 (5'-TGCCTACAAGCATGAAATGG-3') and *inv*A2 (5'-AAACTGGACCACGGTGACAA-3') that amplify a fragment of 457 bp. Amplification products were separated on 1.2% horizontal agarose gels (for *spv*) or 1.5% gels (for *inv*), stained with ethidium bromide, and visualized under UV light using a gel-documentation system (BioRad, MuK nchen, Germany). A 100 bp DNA ladder was used as DNA size standard.

Plasmids were prepared by using a rapid procedure as described by Martie *et al.* [28]. The Plasmid sizes were determined using *Escherichia coli* V517 as the standard [20].

Results

Isolation and subtyping of Salmonella isolates

Sixteen strains belonging to *Salmonella* species were isolated and serotyped in the laboratory and confirmed at the Pasteur Institute in Brussels, Belgium. Those deriving from humans were serotyped as *S.* Kottbus, *S.* Typhi, *S.* Indiana, and *S.* London, while those from food were serotyped as *S.* Hadar, *S.* Corvallis, *S.* Mbandaka, *S.* Typhi, *S.* Ouakam, *S.* Tm var. cop, *S.* Virchow and *S.* Altona.

The distribution of *Salmonella* analyzed in this study is shown in Table 1.

Table 1. Prevalence of *Salmonella* isolated from humans and food.

***Salmonella* isolated**	**Number of strains**	**Sources**
Kottbus	1	Human
Indiana	1	Human
London	1	Human
Typhi	2	Human Food
Hadar	1	Food
Corvallis	2	Food
Mbandaka	2	Food
Ouakam	2	Food
Tm var Cop	1	Food
Virchow	2	Food
Altona	1	Food

Drug resistance

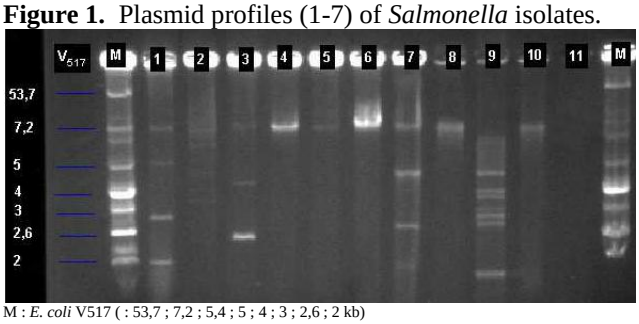
The pattern of resistance of *Salmonella* analyzed in this study is shown in table 2. The highest rates of resistance were observed for furan (37.5%), ticarcillin (31.25%), nalidixic acid (25%), cefalotin (18.75%), and amoxicillin (37.5%), while most expressive intermediate resistance was observed for amoxicillin-clavulanic acid (18.75%) and gentamicin (18.75%). No resistance was observed to amikacin and cefoxitin.

The resistance patterns of *Salmonella* studied in this work were verified in 16 isolates grouped in 12 different patterns. Resistance to more than one drug was seen in 9 isolates (Table 3).

Plasmid profile and phage typing

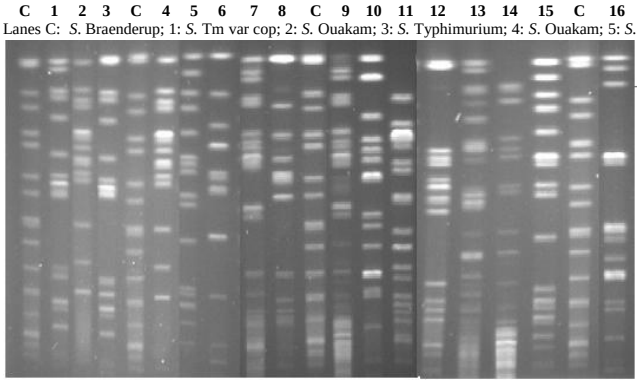
The *Salmonella* isolated showed six different plasmid profiles; the sizes of the plasmids obtained ranged from 2 kb to 7.2 kb (Figure1). Nearly all the isolates harbored a plasmid of approximately 7.2 kb. Table 3 shows the distribution of plasmid types and the molecular weights of the different plasmids for the sixteen strains included in the study. Plasmids of 2.1- 2.8- 5.7 and 7.2 kb were present in RDNC/P12 while a plasmid of 7.2 kb was present in DT104L/ad and PT8. Five serotypes (*S.* Ouakam, *S.* Tm var cop, *S.* Virchow, *S.* Altona, *S.* Corvallis) of *Salmonella* did not show plasmids, thus limiting the potential of this technique for epidemiological studies (Table 3).

Figure 1. Plasmid profiles (1-7) of *Salmonella* isolates.



M : *E. coli* V517 (: 53,7 ; 7,2 ; 5,4 ; 5 ; 4 ; 3 ; 2,6 ; 2 kb)
Lanes 1: *S.* Hadar, 2: *S.* Corvallis, 3: *S.* Mbandak*a*, 4: *S.* Ouakam 5: *S.* Typhi, 6: *S.* Indiana, 7: *S.* London, 8: *S.* Typhi 9: *S.* Kotbus, 10: *S.* Virchow 11: *S.* Tm var cop
Human: 5 – 6 – 7 – 9.
Food: 1 – 2 – 3 - 4 - 8 – 10 – 11

Figure 2. Pulsed field gel electrophoresis (PFGE) patterns for *Xba*I-digested genomic DNA of *Salmonella* strains obtained from human and food with *S.* Braenderup standard.



Lanes C: *S.* Braenderup; 1: *S.* Tm var cop; 2: *S.* Ouakam; 3: *S.* Typhimurium; 4: *S.* Ouakam; 5: *S.* Virchow; 6: *S.* Altona; 7: *S.* Corvallis; 8: *S.* Kottbus; 9: *S.* Corvallis; 10: *S.* Indiana; 11: *S.* Typhi; 12: *S.* Hadar ;13: *S.* Mbandaka; 14: *S.* Mbandaka; 15: *S.* London; 16: *S.* Virchow.

The relationship between plasmid profiles, antibiotype and lysotype patterns of *Salmonella* isolated in this study is shown in table 3. Some isolates have identical serotypes and sources but differ in plasmid profiles and antibiotypes, as in the case of the patterns (F,G) – (I,J) – (K,L). In contrast, other isolates of different serotypes show the same antibiotypes as in the case of the patterns J and L.

ERIC PCR profile

The multiple DNA fragments generated with ERIC1,2 primers were composed of three to nine bands ranging in sizes between 80 and 2000 bp (Figure 3). Visual comparison of the ERIC-PCR banding patterns of all *Salmonella* isolates revealed 8 distinct ERIC polymorphic bands. The food isolates that have the same serotype as *S.* Corvallis, *S.* Ouakam, *S.* Mbandaka and *S.* Virchow showed different ERIC profiles.

PFGE

Electrophoresis of *Xba*I-digested genomic DNA from the 16 isolates of *Salmonella* showed nine different macrorestriction profiles (Figure 2). *Xba*I profiles typically had 11 to 30 restriction fragments. PFGE fingerprinting profiles showed that the food isolates that have the same serotype as *S.* Corvallis *S.* Ouakam and *S.* Virchow share the same PFGE profile.

INV PCR

Examination of *Salmonella* for *inv* A gene was detected in all the strains (data not shown). *spv*R gene was not detected in all *Salmonella* isolates.

Table 2. General percentages of antimicrobial resistance among *Salmonella* isolated from humans and food.

	Antimicrobial resistance (%)													
	AMX	F	TIC	CEF	GEN	TOB	NAL	NOR	CIP	CAZ	AMC	AMK	FOX	CTX
Sensitive *S. enterica*	62.5	62.5	68.75	75	81.25	100	75	87.5	87.5	93.75	81.25	100	100	93.75
Intermediate *S. enterica*	0	0	0	6.25	18.75	12.5	0	0	0	6.25	18.75	0	0	6.25
Resistant *S. enterica*	37.5	37.5	31.25	18.75	0	0	25	12.5	12.5	0	0	0	0	0

amoxicillin, AMX ; furan, F ; ticarcillin, TIC ; cephalothin, CEF ; gentamicin, GEN ; tobramycin, TOB ; nalidixicacid, NAL ; norfloxacin, NOR ; ciprofloxacin,CIP ; ceftazidime, CAZ ; amoxicillin-clavulanic acid, AMC ; amikacin, AMK ; cefoxitin, FOX ; cefotaxime, CTX ; ampicillin, AMP.

Discussion

Some preventive measures against *Salmonella spp*. have been implemented in Morocco. However, salmonellosis is still a global challenge to public health. Epidemiological investigations of these pathogens provide important information about means to control and prevent them in the future. Therefore detailed epidemiological information about this pathogen using molecular-based methods is especially valuable. Numerous published papers examine the relationship between endemic *Salmonella* strains or between isolates involved in outbreaks [30]. In this study, we investigated the suitability of ERIC-PCR fingerprinting to discriminate among *Salmonella* isolates. Phage typing, PFGE and antibiotic resistance profiling were also used to assess relationships between *Salmonella* isolated from human and food samples in northern Morocco.

A total of 16 *Salmonella* strains from different serovars were isolated from human and food. The isolated strains were serotyped and eleven different serotypes were found. The serotype isolates were *S.* Kottbus, *S.* Indiana, *S.* London, and *S.* Typhi from human, and *S.* Typhi, *S.* Hadar, *S.* Corvallis, *S.* Mbandaka, *S.* Ouakam, *S.* Tm var cop, *S.* Virchow, *S.* Altona from food. In Morocco, there is no relevant data about *Salmonella* in humans. In 1998, the National Institute of Hygiene published data on the the relative frequency of different serotypes of *Salmonella* isolated from patients, showing that *S.* Enteritidis was the most frequent serotype isolated (60% in 1995, 76,6% in 1996 and 50% in 1997). This serotype was followed by *S.* Typhi, *S.* Arizona, *S.* Typhimurium, *S.* Albany, and *S.* Papuana [31]. The data presented in this report suggest that the food serotypes of *Salmonella* were more diverse than the human serotypes, although the *S.* Enteritidis serotype remains the most frequent. Unfortunately, these data concern only the capital of Morocco and reinforce the need for similar studies elsewhere in Morocco. In other countries such as Tunisia, the top three frequently isolated serotypes over the course of 11 years (1994 to 2004) were *S.* Enteritidis (25.5%), *S.* Anatum (14%), and *S.* Corvallis (13.2%), identifying Tunisia as an endemic area for these serotypes. Among human isolates, *S.* Enteritidis was the most common serotype, accounting for 24% of all isolates. Among nonhuman isolates, *S.* Anatum (28%), *S.* Enteritidis (69%), and *S.* Corvallis (17.3%) were reported as the first common serotypes for food, animal and environmental samples, respectively [32]. In Libya, nine different serotypes of *Salmonella* were identified: 6 *S.* Saintpaul, 2 *S.* Newport, and 1 *S.* Kottbus [27]. In Togo (Lome), the main serotypes identified, were 147 *S.* Typhi (44.3%), 97 *S.* Typhimurium (29.2%), and 74 *S.* Enteritidis (22.3 %) [33].

Several investigators have used the antimicrobial susceptibility typing of *Salmonella* strains for epidemiological purposes. The isolates resistant to four or more separate classes of antimicrobials were defined as multidrug resistant [7]. The incidence of resistance (*i.e.* resistance to two drugs) and multidrug-resistance (*i.e.* resistance to four or more drugs) of all *Salmonella* strains isolated is presented

in table 3. Several investigators reported that resistance to different antimicrobial agents was mediated by a large plasmid. This plasmid was not found in our strains; however, this observation may not exclude an epidemiological relationship between all isolates because plasmids are unstable genetic elements that can be readily lost or acquired [34].

Phage typing has been used traditionally as a first means of subdivision within serotype *S.* Enteritidis. It has been reported that this technique was the most useful marker for distinguishing clonal groups of *Salmonella* when compared to plasmid analysis, biotyping, and antibiotic susceptibility patterns [9]. However, this system presents some limitations. Not all organisms can be assigned to recognized types, phage conversions are possible [35], and the method requires access to special reagents available only in reference laboratories. In our study the phage typing was done just for three serotypes, *S.* Hadar, *S.* Typhi and *S.* Virchow, because of these limitations. Phage type DT104, which is widely distributed in Europe and North America, was detected in the present study. Further discrimination among strains can be achieved when the phage typing is associated with PFGE analysis [36]. Phage typing discriminated four types that showed multi-resistance to drugs for DT104L/ad and PT20 (table 3). For most isolates, phage typing correlated with biotyping and antibiotyping. It is obvious that phage type may be modified by type phage determining plasmids because acquisition of a plasmid may partially restrict the susceptibility to the typing bacteriophage.

The *Salmonella* isolated harbored different plasmids, showing a genetic difference, but none of them harbor the large plasmid described in the literature and implicated in mediation resistance. Several studies have shown the stability of plasmid profile analysis of *Salmonella* species [37].

ERIC sequence is a short interspersed repetitive consensus sequence originally found in *E. coli* and *S.* Typhimurium. Each of these sequences is targeted by outward-facing primers in PCR by a technique called ERIC-PCR, which discriminates closely related strains. Earlier, ERIC-PCR has been successfully used to discriminate between closely related strains of *Enterobacter aerogenes* and *Escherichia coli* [38]. Though limited in number, there are earlier reports that demonstrate the efficiency of ERIC-PCR to discriminate different *Salmonella* serotypes [39]. This is in accordance with our results for human strains where the ERIC profiles correspond to the serotypes described. Organisms showing identical fingerprinting patterns are considered to be genetically related; however, these relationships were

Table 3: Relationship between antibiotypes and plasmid profiles of Salmonella strains.

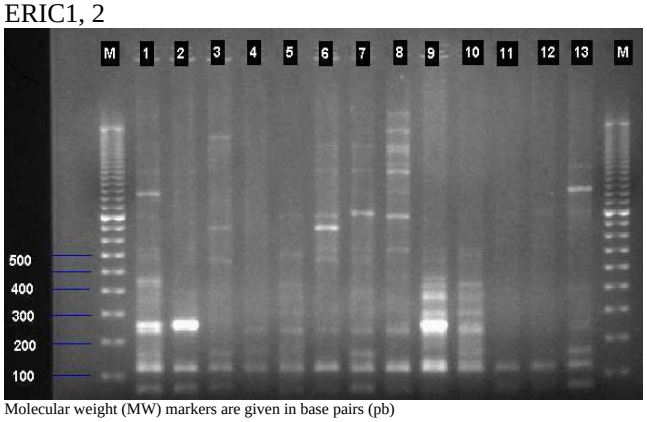
Number of isolates for each pattern	Pattern	Serotype	Source	Antibiotype	Approximate plasmid sizes (Kb)	Lysotype
1	**A**	Indiana	H	AMX,TIC	7.2	ND
1	**B**	Kottbus	H	AMX,TIC,NAL, AMP,TET	2- 2.7-2.8- 3- 4.8- 5.1- 5.5- 7.2- (enter7.2-54)	ND
1	**C**	London	H	AMX,TIC,AMP,TMP,SSS,SXT	2.1- 2.8- 5.5- 7.2	ND
1	**D**	Typhi	H	susceptible	7.2	ND
1	**E**	hadar	F	NAL	2.1- 2.8- 5.7- 7.2	RDNC/P12
1	**F**	Virchow	F	F	7.2	PT8
1	**G**	Virchow	F	AMX,TIC,CEF, F, AMP,TMP,SPE,CHL,GEN,KAN, STR,SSS,SXT	0	PT20
1	**H**	Typhi	F	AMX,TIC,AMP,SPE,CHL,STR,SSS	7.2	DT104L/ad
1	**I**	Corvallis	F	CEF,F	0	ND
1	**J**	Corvallis	F	F,NAL,NOR,CIP	5.1- 6- 7.2	ND
1	**K**	Mbandaka	F	AMX,CEF,F	7.2	ND
1	**L**	Mbandaka	F	F,NAL,NOR,CIP	2.6- 5.3- 7.2	ND
1	**M**	Tm var cop	F	susceptible	0	DT104L/ad

H:human ; F: food
amoxicillin, AMX ; furan, F ; ticarcillin, TIC ; cephalothin, CEF ; gentamicin, GEN ; tobramycin, TOB ; nalidixicacid, NAL ; norfloxacin, NOR ; ciprofloxacin, CIP ; ceftazidime, CAZ ; amoxicillin-clavulanic acid, AMC ; amikacin, AMK ; cefoxitin, FOX ; cefotaxime, CTX ; ampicillin, AMP ; TET, tetracycline ; TMP, trimethoprim; sulfamethoxazol, SSS ; SXT, trimethoprim+sulfamethoxazol ; SPE, spiramycin ; CHL, chloramphenicol ; KAN, kanamycin ; STR, streptomycin.
ND: not done

found to depend on the technique applied [39]. This is in contrast with our findings because the strains of human and food *Salmonella* showed different fingerprinting patterns suggesting that these *Salmonella* isolates are not genetically related. On the other hand, serotypes of food *Salmonella* (*S*. hadar, *S*. Corvallis, *S*. Mbandaka, *S*. Ouakam, *S*. Tm var. cop), have the same ERIC profile, leading to the conclusion that these strains are genetically related.

ERIC-PCR differentiated eight profiles for the sixteen *Salmonella* isolates. This method has the advantages of being simple, accurate, fast, and powerful for the genomic typing of bacterial strains. However, it was shown that ERIC-PCR was better able to differentiate between *Salmonella* strains isolated from human and food sources in this study (Figure 3).

Figure 3. PCR DNA fingerprints generated with primer ERIC1, 2



Molecular weight (MW) markers are given in base pairs (pb)
Lanes: 1: *S.* Hadar; 2: *S.* Corvallis; 3: *S.* Mbandaka; 4: *S.* Virchow; 5: *S.* Ouakam; 6: *S.* Virchow; 7: *S.* Altona; 8: *S.* Indiana; 9: *S.* Typhi; 10: *S.* London; 11: *S.* Typhimurium; 12: *S.* Mbandaka; B 13: *S.* Kottbus.
Rq: *S.* Hadar from food have the same profile as *S.* Corvalis; *S.* Ouakam have the same profile as *S.* Mbandaka; and *S.* Tm var cop have the same profile as *S.* Ouakam.
Human: 8 - 9 -10 - 13
Food: 1 – 2 – 3 – 4 – 5 – 6 - 7- 11 – 12

PFGE is the current gold standard method used to differentiate strains of bacterial pathogens of public health significance to assess the epidemic spread of infectious diseases in hospitals and to trace food-borne outbreaks. Although PFGE is reproducible and discriminatory, some strains of *Salmonella* cannot be typed by PFGE [22]. Only *Xba*I was used in this study, as it is more discriminative and cheaper and has been shown to be a useful restriction enzyme for *Salmonella* spp [40].

In our study, all *Salmonella* isolates were analysed by PFGE to compare how these strains are genetically related. PFGE analysis of 16 isolates of *Salmonella* serotype from human and food showed nine distinct PFGE patterns (Figure 2). The results of PFGE are almost the same as those obtained by ERIC, with the difference lying in the differentiation between the strains of the same serotypes for food isolates. The strains of the same serotype that have different ERIC patterns have the same PFGE patterns, suggesting that this technique indicates that these strains are genetically related.

Several genotypic and phenotypic methods were used in this investigation aimed at determining the reliability of these methods, thus showing good discriminatory power for the typing of *Salmonella* isolates. In this study, the isolates that were discriminated into nine by PFGE types were discriminated into six by plasmid profiles. Thus, the ERIC-PCR, when compared with others (PFGE, plasmid profile) was found more reproducible and showed that ERIC-PCR could be more discriminating in typing *Salmonella* as it allowed differentiation of the 16 *Salmonella* strains into 8 types.

Even though the PFGE technique revealed 9 profiles, PFGE has the disadvantage of being time consuming, involving costly reagents, and requiring specialized equipment. Of the three molecular methods compared, plasmid profiling was clearly the least discriminatory method for the typing of *Salmonella*. In general, the molecular typing of *Salmonella* strains showed that most of the human isolates were different from the nonhuman isolates.

The invasion gene operon, *inv*A is essential for full virulence in *Salmonella* and it is thought to trigger the internalization required for invasion. Our PCR results indicate all *Salmonella* strains tested by PCR were positive for the *inv*A gene (Figure 1). A similar result was reported in a study where 245 *Salmonella* isolates from poultry products, wastewater, and human sources contained the *inv*A gene [24].

The majority of *Salmonella* virulence genes are clustered in chromosomal regions called *Salmonella* pathogenicity islands. However, genes harbored on large virulence plasmids (SAPs), including the virulence-associated locus termed *spv*, might play a role in the multiplication of intracellular *Salmonella* [41]. The gene *spv*R targeted in our study encodes a positive regulatory protein essential for the expression of the other *spv* genes [42]. In this study, all the isolates were negative for *spv*R, indicating that this gene may not be essential to the development of food-borne illness in humans.

Conclusion

This study shows *Salmonella* contamination in humans and in food in northern Morocco. The strains

of *Salmonella* isolated from food sources exhibited six multidrug resistance profiles. The AMX, TIC, AMP, SPE, CHL, STR, SSS profiles were typically associated with phage type DT104L/ad and the AMX, TIC, CEF, F, AMP, TMP, SPE, CHL, GEN, KAN, STR,SSS ,SXT profiles were associated with phage type PT20. In the absence of molecular epidemiological studies in northern Morocco, no information on when exactly the multi-resistant *Salmonella* emerged in Morocco is available.

Among phenotypic methods, phage typing and antibiotyping remain interesting for use in the study of *Salmonella*. Among genetic methods, ERIC typing seems to be well adapted to situations in which rapid comparison and high-quality discriminatory methods for the strains of *Salmonella* are required. PFGE is a good tool for discrimination between strains of *Salmonella* and it can be used as a confirmatory method.

The conclusions made from this study are from a limited number of isolates. It would be desirable to investigate a greater population of *Salmonella* isolates for this purpose.

Acknowledgements

This work conducted and supported by grants from the Pasteur Institute of Morocco in Tangier. We express our gratitude to Dr Ali Madini, Dr. Sarir Majdi, Dr. Naima, Dr. Touba Rajae, Dr. Said bouda, Dr. Mohammed Hamdane, and Dr Mohammed Akad for their valuable help in achieving this study. Thanks go also to Dr Noredine Dersi and Fatna Bourjilat from the Pasteur Institute of Morocco in Casablanca. PFGE and phage typing were performed with the help of the Pasteur Institute in Brussels, and the Reference Laboratory of Salmonellae and shigellae of Belgium.

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Conflict of interest: No conflict of interest is declared.