Epidemiological Analysis of *Salmonella* Enterica Serovar Typhimurium and Serovar 1,4,[5],12:i:- Isolates Determined by Pulsed Field Gel Electrophoresis and Antibiotic Susceptibility: Comparison of Isolates from Broiler Chickens, Humans and the Environment in Reunion Island

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Abstract: *Salmonella enterica* ssp. *enterica* is a leading cause of bacterial food-borne disease outbreaks worldwide and is also an economic burden particularly in Reunion Island because its population consumes large amounts of chicken and cooks 100% chicken sausages (35 kg per capita per year).

The aim of this study was to investigate the epidemiology of *Salmonella* Typhimurium and *Salmonella* 1,4,[5],12:i:- from broiler chickens, humans and the environment by using pulsed field gel electrophoresis (PFGE) and antibiotic susceptibility and to assess the significance of broiler chicken meat as a source of human infection.

A total of 157 *Salmonella* Typhimurium and 19 *S.* I 4,[5],12:i:- were collected and isolated from broiler chickens, humans and the environment between October 2007 and January 2009. The PFGE of Xba1 digested chromosomal DNA gave 30 distinct profiles for *Salmonella* Typhimurium and *S.* 1,4,[5],12:i:-. *Salmonella* Typhimurium was characterized by a main pulsotype (B54) and accounted for 32% of all isolates. This pulsotype included isolates from many sources such as broiler chickens, poultry houses, slaughterhouses, other animal species (ducks, pigs and rodents) and humans, suggesting that it had already colonized every step of the food chain. Antibiotic susceptibility tests showed that most isolates were resistant to ampicillin, streptomycin, sulfonamides and tetracycline.

The similarity of PFGE profiles of isolates from various sources and particularly from poultry and humans underlined possible transmission of *Salmonella* from contaminated broiler meat, but most of the isolates remained drug-sensitive.

Significance and impact of study: Efforts are needed to eliminate *Salmonella* from poultry meat destined for human consumption. This study has also shown the importance of monitoring antimicrobial resistance in bacteria associated with animals and humans.

Keywords: Antibiotic susceptibility, broiler chickens, humans, PFGE, reunion Island, salmonella

INTRODUCTION

"Salmonella is a leading cause of bacterial food-borne disease outbreaks in temperate countries [1] and is also a public health concern in tropical countries" [2,3].

"The most commonly implicated foods in outbreaks of human salmonellosis are those of animal origin" [4]. "Most of these infections have been attributed to the consumption of poultry meat and eggs" [5]. "*Salmonella enterica* subsp. *enterica* serovar Typhimurium is one of the most common serovars isolated from humans, animals and food in Europe and the United States [6,7]. In France, *Salmonella* Typhimurium, *S.* Enteritidis, and *S.* 1,4,[5],12:i:- were the serovars most frequently isolated in 2008 at 46%, 19% and 4% of clinical isolates, respectively. Furthermore, during the last decade *Salmonella* 1,4,[5],12:i:- has emerged around the world [8], and this isolate could be a monophasic isolate of serotype Typhimurium.

Salmonella causes diverse disease syndromes ranging from asymptomatic colonization to severe intestinal illness [9]. Antimicrobial therapy may be needed; fluoroquinolones and β-lactams are the antibiotic drugs of choice. Nevertheless, global outbreaks of multidrug-resistant *Salmonella* have been reported, particularly for *S. Typhimurium*. Resistance to fluoroquinolones and extended spectrum cephalosporins is still growing in the

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European Union, Africa [10] and Asia, for example in Japan where a high level of fluoroquinolone-resistant isolates was first identified in 2000 [11]. Resistance is of utmost importance to worldwide public health, and controlling antimicrobial resistance is important to limit the transfer of resistant *Salmonella* from animals to humans.

Salmonella from poultry have been studied all over the world but no epidemiological study had previously been undertaken in Reunion Island. This island is located in the Indian Ocean, east of Madagascar and west of Mauritius. Reunion is an administrative region of France. Chicken meat production is locally consumed (providing 66% of chicken consumption, with 33% from frozen chicken imported from France). Contamination of chicken with *Salmonella* is both a public health and an economic concern especially since the population of Reunion Island consumes a large amount of chicken (35 kg per capita per year) and cooks 100% chicken sausages.

The aim of this study was to investigate the molecular epidemiology of *S. Typhimurium* and *S.* 1,4,[5],12:i:- from broiler chickens, humans and the environment using pulsed field gel electrophoresis (PFGE) and antibiotic susceptibility and to assess the significance of broiler chicken meat as a source of human infection in Reunion Island.

MATERIALS AND METHODS

Sample Collection

Between October 2007 and January 2009, a total of 157 *Salmonella enterica* serovar Typhimurium and 19 *Salmonella* 1,4,[5],12:i:- isolates were collected and isolated on Reunion Island from broiler chickens, poultry farms, slaughterhouses, other animals (pigs, ducks, turkeys and rodents) and humans (Table **1**).

The poultry isolates all came from live broiler chickens (faeces and litter) and from carcasses. The environment isolates came from farm environment (changing room, wall and equipment, poultry house surroundings, litter beetles (tenebrionidae), trucks (wheels) and rodents, from abattoir environment (transport crates, scalding water, defeathering and evisceration stages, trussing and cutting tables, utensils and from sausages) and from other animals (ducks, turkeys, geese…).

Isolates from pigs were acquired from a previous study [12] and some isolates from ducks and turkeys were obtained from the local veterinary laboratory.

For humans, isolates were received from the main hospital in the south of Reunion Island, private laboratories and from the Pasteur Institute in Paris, France.

Microbiological Methods

Salmonella strains were isolated by the standard culture method in accordance with NF U47-100:2007 (French Standards Association) as previously described [13]. All *Salmonella* isolates were serotyped according to the Kauffmann-White scheme [14] and the slide agglutination test using *Salmonella* polyvalent O and H antisera in accordance with the Diagnostic Pasteur.

Molecular Typing: RFLP/PFGE

DNA Extraction

The following harmonized protocol was used for the study as described previously [15]. After overnight growth on PCA or nutrient broth, cells were harvested by centrifugation and re-suspended in suspension buffer. The final cell density for plug preparation was 1.3-1.6 at 600nm. Proteinase K was added to the cell suspension followed by mixing lysis of cell suspension 1:1 with SeaKem Gold Agarose. The resultant plugs were washed at least twice in distilled water and four times in TE buffer.

Enzymatic Digestion

The genetic typing was carried out using the RFLP-PFGE PulseNet protocol [16] and total DNA was digested with one restriction enzyme X*baI* (Roche Applied Science). The obtained fragments were separated in 1% agarose (SeaKem Gold Agarose) gels using the CHEF-DR-III system (Bio-Rad Laboratories, USA).

Electrophoresis

Electrophoresis was carried out with 0.5X TBE buffer at 6 V/cm and 14°C. The running time was 20 hours and the pulse ramp time was 2.2–63.8 s. *Salmonella enterica* serovar Braenderup H9812 was used as a molecular weight marker.

Gels were visualized on a UV transilluminator and photographs were captured using a digital imaging system (Video gel doc system, Bio-Rad). Fragment restriction patterns were analysed by BioNumerics software (Applied Maths, Sint Marteen, Belgium), performed using UPGMA (unweighted pair-group method with an arithmetic mean) and a Dice similarity coefficient [17] with a tolerance index of 5%, a position tolerance setting of 1% and an optimization setting of 1% generating a dendrogram. Fragments smaller than 30 kb were disregarded in accordance with the PulseNet guidelines for standardization [18].

Discrimination Power

Discrimination power was calculated by determining the Simpson discrimination indices (D) as per Hunter [19]. These values represent the probability that two distinct isolates will be ranged into different typing groups.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility was tested by the disk diffusion method following the CLSI guidelines (Clinical and Laboratory Standards Institute, 2008).

The isolates were tested for their susceptibility to ampicilin (A; 10 µg), amoxicillin–clavulanic acid (AMC; 20/10 µg), cefotaxime (CTX; 30 µg), chloramphenicol (C; 30 µg), cephalothin (CF; 30 µg), ceftazidime (CAZ; 30 µg), cotrimoxazole (SXT; 1.25/23.75 µg), sulfonamides –

Table 1. Type of samples and PFGE pattern of *Salmonella* Typhimurium and *Salmonella* S. 1,4,[5],12:i:- collected from broiler chickens, farms and the slaughterhouse, from other animals, humans and from some foodstuffs (Reunion Island, 2007-2009, 176 isolates).

Type of Samples			Number of Isolated Strains (%)	PFGE Pattern (Number of Each Pulsotype)
Broiler chicken	Broiler chicken	Faeces and litter	33 (19)	B50(8);B52(4);B54(12);B59(1);B61(1);B63(1); B64(1);B70(1);B75(1);B77(3)
		Neck skin	3 (2)	B54(3)
		Caeca	5 (3)	B54(2);B62(1);B73(1)
		Carcasses	19 (11)	B54(5);B62(4);B69(1);B73(6);B74(1);B77(2)
Environment	Broiler farm	Wall and equipment	2 (1)	B55(1);B67(1)
		Sas	3 (2)	B54(1);B62(2)
		Surroundings	2 (1)	B58(1);B69(1)
	Slaughter house	Transport crate	3 (2)	B50(2);B62(1);B73(1)
		Scalding water	3 (2)	B50(2);B51(1)
		Before defeathering	7 (4)	B60(1);B62(2);B69(1);B73(2);B77(1)
		After defeathering	4 (2)	B50(1);B54(1);B69(1);B73(1)
		Before trussing table	3 (2)	B73(1);B77(2)
		After trussing table	6 (3)	B54(2);B69(1);B73(2);B77(1)
		Evisceration	6 (3)	B54(4);B73(1);B77(1)
		Cutting tables	5 (3)	B54(1);B72(2);B73(2)
		Utensils	1 (1)	B77(1)
	Other animals	Pig	11 (6)	B54(4);B61(2);B64(2);B69(1);B72(1)
		Duck	10 (6)	B51(5);B52(1);B54(1);B56(3)
		Rooster	4 (2)	B54(4)
		Turkey	5 (3)	B54(3);B62(1);B71(1)
		Guinea fowl	3 (2)	B49(1);B53(1);B62(1)
		Goose	1 (1)	B61(1)
		Rodent	1 (1)	B54(1)
Human	Human	Human	33 (19)	B49(1);B50(1);B53(3);B54(13);B56(1);B57(1); B61(2);B62(2);B64(1);B65(1);B66(1); B68(2);B69(1);B71(2);B78(1)
Food stuff		Sausage	1 (1)	B73(1)

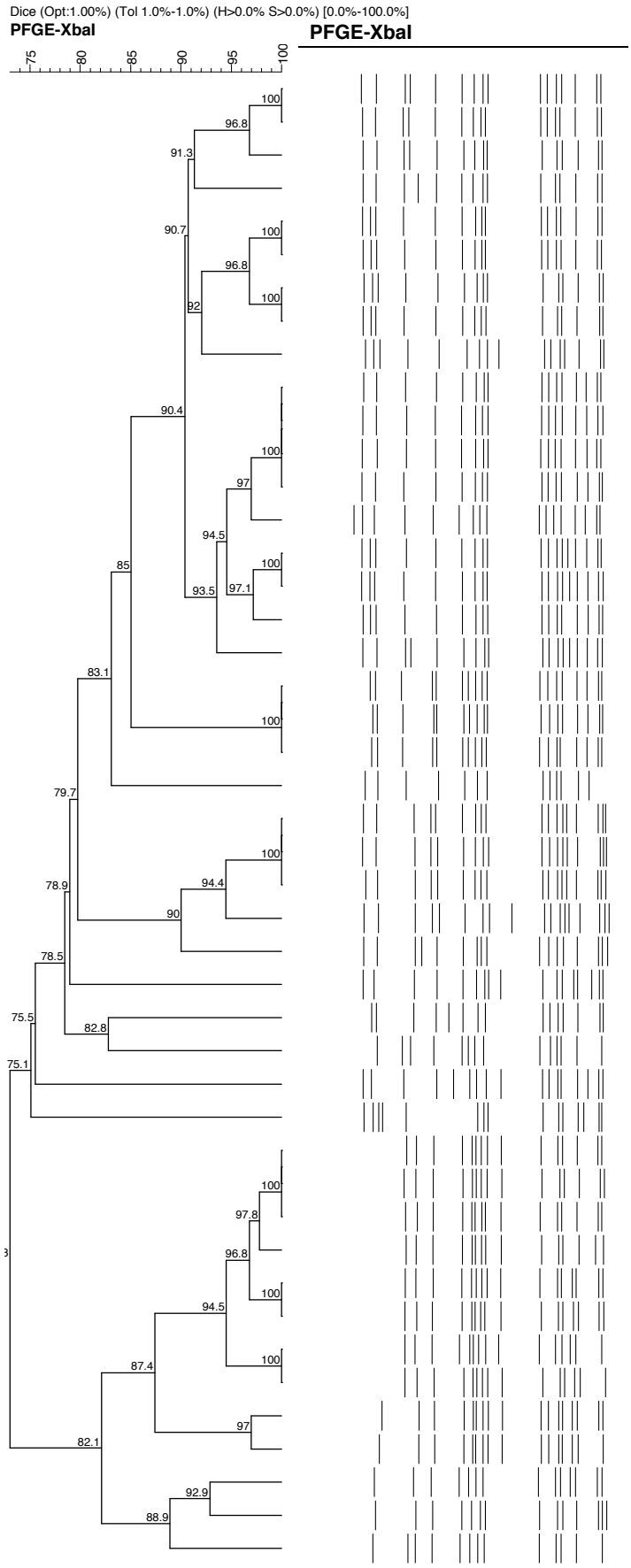
NCCLS (Su; 300 µg), gentamicin (Gm; 10 µg), streptomycin (S; 10 µg), kanamycine (K; 30 µg), tetracycline (T; 30 µg), colistine (Cs; 10 µg), nalidixic acid (Na; 30 µg), ofloxacine (Ofx; 5 µg) and enrofloxacine (Enr; 5 µg). *Escherichia coli* (ATCC25922) was used as control strain.

RESULTS

PFGE and Genetic Diversity

The genotyping of 157 isolates of *Salmonella* Typhimurium and 19 isolates of *S.* 1,4,[5],12:i:- was carried out by PFGE using X*ba1* as macrorestriction enzyme. Digestion of DNA revealed 30 profiles (B49 to B78) (Fig. **2**). The discriminatory ability (D value) of the method was 0.86 for the entire panel. Analysis by BioNumerics software showed an overall similarity of 75% with stable patterns consisting of 14-18 fragments.

The genetic relatedness of the PFGE profiles for *Salmonella* Typhimurium and *S.* 1,4,[5],12:i:- showed 5 clusters (Fig. **1**) but *S.* 1,4,[5],12:i:- was only found in clusters 4 and 5: the first cluster (17.3% of the isolates) consisted of 6 profiles (B49, B50, B51, B52, B53 and B59): 12 isolates from chicken, 4 from the slaughterhouse (2 from scalding water, 1 from the defeathering stage and 1 from transport crates), 8 from other animals (2 turkeys, 6 ducks), 5 from humans and 2 from sausages. The second cluster (35.7%) consisted of 5 profiles (B54, B55, B56, B57 and B58) from 24 chicken isolates, 11 from broiler farms and slaughterhouses (outdoor area, changing rooms, walls and equipment, transport crates, scalding water, evisceration and cutting table); 14 from other animals (rodent, turkey, wild bird and duck isolates) and 15 from humans; the third cluster (12.8% of the isolates) comprised 3 profiles (B62, B63 and B64): 11 from chickens, 5 from farm and abattoir



Key	XbaI	Genre	Serotype	Origin
IH-241	B53	Salmonella	Typhimurium	Human
IH-340	B53	Salmonella	Typhimurium	Poultry
IH-294	B52	Salmonella	Typhimurium	Poultry
IH-277	B51	Salmonella	Typhimurium	Poultry
IH-342	B49	Salmonella	Typhimurium	Poultry
IH-409	B49	Salmonella	Typhimurium	Human
IH-262	B50	Salmonella	Typhimurium	Poultry
IH-396	B50	Salmonella	Typhimurium	Human
IH-299	B59	Salmonella	Typhimurium	Poultry
IH-212	B54	Salmonella	Typhimurium	Poultry
IH-235	B54	Salmonella	Typhimurium	Rodent
IH-242	B54	Salmonella	Typhimurium	Human
IH-288	B54	Salmonella	Typhimurium	Poultry
IH-231	B55	Salmonella	Typhimurium	Poultry
IH-237	B56	Salmonella	Typhimurium	Human
IH-422	B56	Salmonella	Typhimurium	Poultry
IH-286	B57	Salmonella	Typhimurium	Human
IH-247	B58	Salmonella	Typhimurium	Poultry
IH-291	B61	Salmonella	Typhimurium	Poultry
IH-351	B61	Salmonella	Typhimurium	Poultry
IH-408	B61	Salmonella	Typhimurium	Human
IH-269	B60	Salmonella	Typhimurium	Poultry
IH-233	B62	Salmonella	Typhimurium	Poultry
IH-245	B62	Salmonella	Typhimurium	Human
IH-276	B62	Salmonella	Typhimurium	Poultry
IH-312	B63	Salmonella	Typhimurium	Poultry
IH-411	B64	Salmonella	Typhimurium	Human
IH-419	B77	Salmonella	Typhimurium	Poultry
IH-397	B65	Salmonella	Typhimurium	Human
IH-410	B66	Salmonella	Typhimurium	Human
IH-413	B68	Salmonella	Typhimurium	Human
IH-258	B67	Salmonella	Typhimurium	Poultry
IH-203	B69	Salmonella	S.I 4,12:i:-	Poultry
IH-315	B69	Salmonella	Typhimurium	Pork
IH-403	B69	Salmonella	Typhimurium	Human
IH-421	B70	Salmonella	Typhimurium	Poultry
IH-395	B71	Salmonella	Typhimurium	Human
IH-423	B71	Salmonella	Typhimurium	Poultry
IH-224	B72	Salmonella	Typhimurium	Poultry
IH-317	B72	Salmonella	Typhimurium	Pork
IH-249	B73	Salmonella	Typhimurium	Poultry
IH-254	B74	Salmonella	Typhimurium	Poultry
IH-337	B75	Salmonella	Typhimurium	Poultry
IH-399	B78	Salmonella	Typhimurium	Human
IH-319	B76	Salmonella	S.I 4,12:i:-	Poultry

Fig. (1). Dendrogram showing the cluster analysis of PFGE Xba1 patterns from 161 isolates of *Salmonella* Typhimurium and 18 Salmonella 1,4,[5],12:i:- generated by BioNumerics software using the UPGMA method.

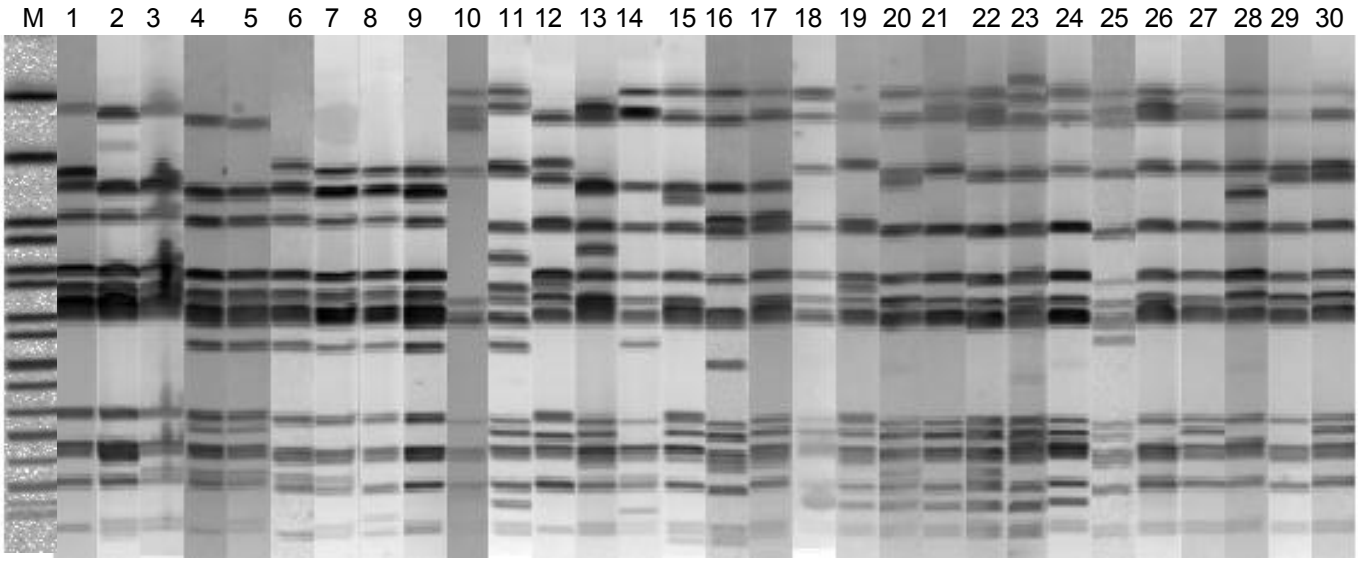


Fig. (2). The PFGE Type for Xba1 digested genome DNA of Salmonella Typhimurium and Salmonella S. 1,4,[5],12:i:- . A total of 176 isolates were analysed; 30 Xba1 PFGE-types were obtained: M:Salmonella Braenderup; lane1:B76; lane2:B78; lane3:B75; lane4:B74; lane5:B73; lane6:B72; lane7:B71; lane8:B70; lane9:B69; lane10:B67; lane11:B68; lane12:B66; lane13:B65; lane14:B77; lane15:B64; lane16:B63; lane17:B62; lane18:B60; lane19:B61; lane20:B58; lane21:B57; lane22:B56; lane23:B55; lane24:B54; lane25:B59; lane26:B50; lane27:B49; lane28:B51; lane29:B52; lane30:B53.

environment (changing rooms, transport crates, defeathering and evisceration stages), 4 from other animals (pigs, turkeys and guinea fowl) and 3 from humans; the fourth cluster (7.8% of the isolates) comprised 4 profiles (B69, B70, B71 and B72): 1 from chicken, 3 from farm and abattoir environment (outdoor area and cutting table), 3 from other animals (pigs and turkey) and 2 from humans for *S. Typhimurium*; for *S.* 1,4,[5],12:i:-, the fourth cluster comprised 1 isolate from chicken, 1 human and 3 isolates from the slaughterhouse environment (defeathering stage and trussing table). Finally, the fifth cluster (10.6% of the isolates) comprised 2 profiles (B73 and B74): 2 isolates from chickens, 4 from the slaughterhouse environment (defeathering and evisceration stages, trussing and cutting tables) and 1 from sausages for *S. Typhimurium*; 6 chicken isolates and 6 isolates from the abattoir environment (transport crates, defeathering stage, cutting and trussing tables) were found for *S.* 1,4,[5],12:i:-.

Antibiotic Resistance Patterns

Salmonella Typhimurium. Among a total of 157 *Salmonella* Typhimurium isolates, 9 (5.5%) were resistant to A, 28 (17.4%) to S, 32 (19.9%) to Su, 4 (2.5%) to SXT and 37 (22.3%) to T. The same results were observed for isolates from the environment or humans (Table **2**). Only 1 isolate from other animals exhibited a resistance against Na.

Salmonella 1,4,[5],12:i:-. Out of 19 isolates, 19 (100%) was resistant to A, S, Su and T.

Antibiotic Resistance Associated with PFGE Pattern

The main resistance pattern associated with most of the pulsotypes (B50, B69, B70, B71, B73, B74, B75 and B76) is A,S,Su,T. We also found two other resistance patterns: A,Su,SXT,T (B62 and B63) and S,Su,T (B54 and B55). Three isolates from pig and human origin yielded the typical multidrug resistant pattern A,C,S,Su,T (Table **3**).

DISCUSSION

In Reunion Island, *S. Typhimurium* is the most prominent serovar in broiler chickens, just as in other parts of the world [20-22]. It appeared that *S. Typhimurium* was able to infect many hosts, including monogastric species, poultry (chicken, duck, turkey, guinea fowl, geese) and pigs, but also small mammals such as rodents [23, 24]. The atypical *Salmonella enterica* 1,4,[5],12:i:- emerged a few years ago in Reunion Island and because of the close genetic relationship, it is certainly a monophasic isolate of *S. Typhimurium* [8]. Many studies have already explained the relationships between serovars S.1,4,[5],12:i:- and Typhimurium through the presence of IS200 by DNA microarray [25,26].

We opted for the method of choice for typing *Salmonella*; pulsed field gel electrophoresis (PFGE) remains the gold standard for *Salmonella* genotyping. Its discriminatory power is good and this method has proved to be highly useful in outbreak situations and has been widely used for *Salmonella* fingerprinting [22,27,28]. Use of PFGE with endonuclease *Xba*1 has been recognized as a precise means for fingerprinting *Salmonella* serovars [29], particularly for *S. Typhimurium* [30].

The strong similarity found between isolates from broiler chickens, humans and the environment indicated a close genetic relationship between avian serovars Typhimurium and 1,4,[5],12:i:- compared to that of isolates from other sources. Previous studies on clonal relationships of *S. Typhimurium* from humans and various other sources showed that isolates of this serovar were clustered into a group with similarity of more than 70%. This observation was in agreement with our findings (75.1% similarity). In spite of their close genetic relationship, it was possible to

Table 2. Antimicrobial resistance for *Salmonella* Typhimurium and 1,4,[5],12:i:- isolates from chickens, the environment, other animals and humans.

	Origin							
	Chicken		Environment		Other animals		Human	
	S. Typhimurium	S 1,4,[5],12:i:-	*S. Typhimurium*	S 1,4,[5],12:i:-	*S. Typhimurium*	S 1,4,[5],12:i:-	*S. Typhimurium*	S 1,4,[5],12:i:-
Ampicilin (Am)	9(5.6)	6(33.3)	7(4.3)	9(50)	6(3.7)	1(5.5)	4(2.5)	1(5.5)
Amoxicilinc lavulanic acid (AMC)								
Chloramphenicol (C)					1(0.6)		3(1.9)	
Ceftazidime (CAZ)								
Cephalotin (Cf°)								
Colistine (CS								
Cefotaxime (CTX)								
Enrofloxacine (ENR)								
Gentamicine (GM)								
Kanamycin (K)								
Nalidixic Acid (NA)					1(0.6)			
Ofloxacine (OFX								
Streptomycine (S)	28(17.4)	6(33.3	17(10.5)	9(50)	15(9.3	1(5.5)	16(10%)	1(5.5)
Sulfonamides (SSS250	32(19.9)	6(33.3)	18(11.2)	9(50)	18(11.2)	1(5.5)	18(11.2)	1(5.5)
Cotrimoxazole (SXT)	4(2.5)		1(0.6)		2(1.2)		3(1.9)	
Tetracyclin (Te)	37(22.3)	6(33.3)	23(14.3)	9(50)	19(11.8)	1(5.5)	19(11.8)	1(5.5)

(): numbers in parentheses represent percentage of resistant isolates for Typhimurium and 1,4,[5],12:i:- respectively.

divide the majority of the avian isolates into five clusters. The first two and the last two clusters were closely related; this suggested, at least, the introduction of two or three different clones that could have been brought in *via* imports of foodstuffs, parent stocks, hatching eggs or one-day-old chicks from France or from south-east Asia [31, 32] in the 1980s.

The main pulsotype (B54) accounted for 29% of all the isolates; it comprised isolates from many sources such as poultry houses, the slaughterhouse, other animal species (ducks, turkeys, pigs or rodents) and humans, suggesting that this pulsotype had already colonized every step of the food chain [33]. This pulsotype also showed a close genetic relationship between the isolates from broilers and those from rodents. As demonstrated by Meerburg and Kijlstra [34], rodents are often implicated in the infection of poultry. Indeed, rodents have been recognized as a vehicle for *Salmonella* [23,35]. In Reunion Island, rodents are a real problem because most of the territory is covered with sugarcane fields, which provide a natural habitat for rodents, and are usually very close to poultry farms [36, 37].

The same pulsotypes of *Salmonella* have been recovered from different animal species; in a tropical island like Reunion where all farms (pig and poultry) are concentrated in a small area, exchange of organic material and pathogens between these farms *via* trucks, employees and technical staff is still possible. Moreover, many farmers rear pigs and broilers at the same time on the same site [12]. A cycle of transmission between these different species could have been instigated and this could explain the close genetic relationship between these *Salmonella* isolates [38].

Most of the chicken isolates (2nd, 4th and 5th clusters) had the same genotype pattern as the environmental isolates from the outdoor areas of poultry farms; this suggested *Salmonella* Typhimurium isolates could persist in the environment even after cleaning and disinfection [39]. This persistence could be explained by failures in decontamination procedures [40]; as an example, poultry manure is often kept outside, with or without protection, to be used as fertilizer for nearby market gardening. But these isolates could also be re-introduced into the poultry farm *via* different routes, such as rodents, or even rainwater; [41] had already demonstrated that *Salmonella* could be disseminated in the soil as a result of substantial rainfall, frequent in tropical climates such as that of Reunion Island.

The same pulsotypes were found in chickens, poultry houses and the slaughterhouse, confirming that the slaughterhouse had been contaminated by infected chickens [33, 42].

The same *S. Typhimurium* PFGE patterns (B54, B62, B64 and B69) observed for poultry and human isolates underlined a possible contamination of humans by chicken as previously described by Nogrady *et al*. [43]. The presence of *S. Typhimurium* in broiler chickens is of considerable importance from the standpoint of public health and particularly in Reunion island where it is the most frequent serovar incriminated in food poisoning [44]. Most of these

Table 3. PFGE and antibiotic susceptibility patterns of *Salmonella* Typhimurium and *Salmonella* S.I 4,12:i:- collected from broiler chickens, from farm and abattoir environment, from other animals, and humans (Reunion Island, 2007-2009, 176 isolates).

PFGE Pattern	Number of Strains Associated (%)	R Type	Origin											
			Broiler Chicken		Human		Broiler Farms		Slaughter House		Other Animals		Foodstuffs	
			S 1,4,[5], 12:i:-	*S. Typhimurium*	S 1,4,[5], 12:i:-	*S. Typhimurium*	S 1,4,[5], 12:i:-	*S. Typhimurium*	S 1,4,[5],12:i:-	*S. Typhimurium*	S 1,4,[5], 12:i:-	*S. Typhimurium*	S 1,4,[5], 12:i:-	*S. Typhimurium*
B49	2(1.1)					1						1		
B50	13(7.4)			6		1				4				2
B50	1(0.6)	A,S,Su,T		1										
B51	5(2.8)											5		
B52	5(2.8)			4								1		
B53	4(2.3)					3						1		
B54	4(2.3)					1		1		2				
B54	1(0.6)	Na,S,Su,T		1										
B54	52(29.5)	S,Su,T		20		12				7		13		
B55	2(1.1)	S,Su,T						1				1		
B56	1(0.6)					1								
B56	1(0.6)	A,Su,Sxt,T										1		
B56	1(0.6)	Na, T										1		
B57	1(0.6)					1								
B58	1(0.6)	S,Su,T						1						
B59	1(0.6)			1										
B60	1(0.6)									1				
B61	1(0.6)					1								
B61	4(2.3)	T				1						3		
B62	13(7.4)			7		1		2		2		1		
B62	6(3.4)	A,Su,Sxt,T		3		1				1		1		
B63	1(0.6)	A,Su,Sxt,T		1										
B64	3(1.7)	A,C,S,Su,T				1						2		
B65	1(0.6)					1								
B66	1(0.6)	C,Su,Sxt,T				1								
B67	1(0.6)							1						
B68	2(1.1)					2								
B69	7(4)	A,S,Su,T	1			1		1	3			1		
B70	1(0.6)	A,S,Su,T	1											
B71	3(1.7)	A,S,Su,T			1	1						1		
B72	2(1.1)	S,Su,T								1		1		
B72	1(0.6)	A,S,Su								1				
B73	18(10.2)	A,S,Su,T	6	1					6	4				1
B74	1(0.6)	A,S,Su,T		1										
B75	1(0.6)	A,S,Su,T		1										
B76	1(0.6)	A,S,Su,T									1			
B77	11(6.3)	T		5						6				
B78	1(0.6)					1								

isolates exhibited the same genetic pattern but showed differences in susceptibility to antibiotic drugs. This variability could be explained by genetic changes; mutation or horizontal transfer, linked for example to the selective pressure of drugs at the farm [45].

Multidrug resistance was generally observed in *Salmonella* Typhimurium [46] but in this study, only three isolates - one from human and two from pigs - exhibited the specific profile ACSSuT. This profile matched the phage type DT104 but it was not the prominent profile in our study. *Salmonella* Typhimurium DT104 has spread in various countries [47, 48] and it could also be present in Reunion Island. Nevertheless, this resistance profile was not found in chicken isolates; this could be explained by different practices in the poultry and pig industries.

Most *Salmonella* Typhimurium and *Salmonella* 1,4,[5],12:i:- isolates were susceptible to all the tested antibiotic drugs in contrast to results observed in mainland France [44]. Most of the isolates from Reunion Island showed resistance to ampicilin, streptomycin, sulfonamides and tetracycline as previously identified [49]. These antibiotic drugs have been the most commonly used antibiotic drugs in animal production in Reunion Island and this explained the frequent occurrence of resistance to these antimicrobial agents [50, 51]; only one *S. Typhimurium* isolate was resistant to nalidixic acid whereas this resistance has been observed frequently in the USA [52], in Japan [53] and in South-East Asia [54].

Analyses using serotyping and more specifically macrorestriction profiling by PFGE with Xba1 showed that no clonal relationship existed between PFGE and antibiotic resistance profiles. "The antimicrobial resistance characteristics could have been acquired by selective pressure of drugs or by horizontal transfer" [55]. It is therefore necessary to investigate veterinary practices to understand the differences between the pig and poultry industries.

This study strongly indicates a close genetic relationship between *S. Typhimurium* and *S.* 1,4,[5],12:i:- isolates from humans and broiler chickens. But poultry meat is not the only source of human *Salmonella* infections however, since the same profiles have been recovered from other animals. And even if the resistance of *Salmonella* to antibiotic drugs remains low, it also highlights the need for continuous surveillance to monitor antimicrobial resistance in bacteria associated with animals and humans.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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