

Prevalence and antibiotic-resistance of *Salmonella* isolated from beef sampled from the slaughterhouse and from retailers in Dakar (Senegal)

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Abstract

A study was made of *Salmonella* contamination in beef sampled from a slaughterhouse and from retailers in Dakar, Senegal. The serotypes as well as antibiotic-resistance patterns of the *Salmonella* isolates were determined.

A total of 435 meat samples (236 from the slaughterhouse, 199 from retailers) were tested. Among them, 275 (63%) were positive for *Salmonella*, 43% (101/236) from the slaughterhouse and 87% (174/199) from the retailers. Furthermore, 97% of the investigated retailers had at least one beef sample contaminated by *Salmonella*.

The 286 *Salmonella* isolates were divided into 51 serotypes. The most prevalent serotypes were *Salmonella bredeney* (25%), *S. muenster* (8%), *S. waycross* (7%), *S. corvallis* (4%) and *S. kentucky* (4%). About 62% of the isolates were resistant to nitrofurans. Resistance rates were lower to streptomycin (22%), sulfamethoxazole (15%), spectinomycin (1%), chloramphenicol (1%), and tetracycline (0,4%) while low-level resistance to quinolones was detected. About 16% of the *Salmonella* strains were multiresistant to two or more antibiotic families. Finally, ten resistance profiles have been identified.

This study shows the huge spread of *Salmonella* in the beef production chain in Dakar, Senegal. Finally, this study provides the very first data about *Salmonella* prevalence in sub-saharian Africa.

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1. Introduction

After malaria and respiratory infections, diarrhoeal diseases are one of the main causes of morbidity and mortality especially for children in developing countries. Among those diseases,

salmonellosis is considered as the most common foodborne disease in developing countries as well as in industrialized ones, although incidence rates vary according to the country (Motarjemi et al., 1995).

The development and the accumulation of resistance to antibiotics in these pathogens are a major issue in public health. It is generally accepted that, in developing countries some multi-resistant *Salmonella* are of animal origin and acquire their resistance in animals before being transmitted to human through

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the food chain (White et al., 2001; Threlfall, 2002). In the United States, a study showed that 20% of 200 samples of ground chicken (35%, $n=51$), beef (6%, $n=50$), turkey (24%, $n=50$) and pork (16%, $n=49$) purchased at three retail store are infected by *Salmonella* (White et al., 2001).

In developing countries, the few surveys available as well as the lack of surveillance network make it difficult to assess the magnitude of these diseases. Very few data exist in particular for the Sahelian area.

The incidence of food-borne infections is poorly known in Senegal, reporting of food-borne outbreaks being not mandatory. It is therefore difficult to estimate the extent of the phenomenon. Nevertheless, periodic epidemics indicate wide spread circulation of the causative organism: cholera in Dakar in 2004, *Salmonella* multi-resistant to antibiotics in 2001 (Cardinale et al., 2001) and a multicentric study undertaken on street food indicate significant contamination (Garin et al., 2002). Various studies carried out in hospitals also highlighted many cases of diarrhoeal syndromes due to *Salmonella* (Lafaix et al., 1979; Cissé et al., 1991, 1993). In a study of *Salmonella* serotypes isolated over 6 years at the paediatric hospital (Cissé et al., 1993), 55% of the isolated strains were non-typhoid. Seventeen percent of the isolated *Salmonella* strains were multiresistant to antibiotics and 8% produced a beta-lactamase.

Analyses carried out at the Food Safety and Environmental Hygiene Laboratory of the Institut Pasteur de Dakar show a high contamination, by fecal peril germs (*Escherichia coli*, *Salmonella*, sulfite-reducing *Clostridium*), of food products (prepared meals, fresh vegetables, cold meals, pastries, dairy products) and in particular a contamination of meat products (butcher's meats) (unpublished data).

In meat production, the leading source of contamination of carcasses by *Salmonella* is the evisceration step at the slaughterhouse (Samuel et al., 1980). In Ethiopia, approximately 20% of camel carcasses were found positive for *Salmonella* at slaughtering. At the retailers' level, 15% of the meat samples were found positive for *Salmonella* in Ethiopia (Ejeta et al., 2004). In Senegal, no data on *Salmonella* prevalence on beef was yet available.

The microbiological quality of meat sandwiches sold on the streets was studied in Dakar, in 1997. The results showed that 52% of the samples were microbiologically nonsatisfactory regarding European microbiological criteria (Garin et al., 2002). Then another study, undertaken in 2000, in the poultry industry highlighted a new *Salmonella* pathogenic strain multiresistant to antibiotics (Cardinale et al., 2001). This strain was isolated in farm stock as well as among hospitalized patients.

Food poisoning due to *Salmonella* in Senegal is a public health issue, but its origins are so far poorly known. A survey carried out for the Farming Department provided the opportunity to obtain data on meat contamination by *Salmonella*, no previous data being available. The high contamination rate raised questions about the rest of the meat production chain and its implication in human infections.

This work describes the prevalence, serotypes and phenotypes of antibiotic-resistance of *Salmonella* strains found in meat sampled at the slaughterhouse and retailers in Dakar, Senegal. This survey provides data for the sub-saharian area.

2. Materials and methods

2.1. Sampling and survey

Bovine carcasses were collected at the only official city slaughterhouse at the end of slaughtering operations, prior to storage in cold room. A total of 236 carcasses were collected at the slaughterhouse. An average of 6 carcasses per slaughtering day (1–10) were randomly collected out of the 100 to 150 animals slaughtered daily. Sample collection was carried out between 7:00 a.m. and 11:00 a.m. (between 7:00 a.m. and 9:00 a.m. for 70% of the samples) during April to July 2003.

In addition, 199 samples from 73 retailers were randomly selected out: 34 samples from 10 modern butcher shops, 83 samples from 170 market stalls, 43 samples from 20 districts retailers, 39 samples from 100 itinerant retailers. The origin of the retailers' meat was the official city slaughterhouse, previously sampled. An average of 8 samples per day was collected between April and August 2004.

Each sample was accompanied by a questionnaire filled out at the time of the collection. At the slaughterhouse, the information collected related to husbandary practices (extensive or intensive), breed, sex, age and animal weight, area of origin, carcass conformation (visual appreciation), and presence of subcutaneous and internal fat. No information regarding the administration of antibiotics was available.

At retailers, information about structure and practices were collected: temperature conditions of the meat transfer from the slaughterhouse to the place of sale (frozen, chilled or room temperature), storage temperature conditions (frozen, chilled or room temperature), cleanliness of the cutting tools, covering materials on the cutting up tables, cleaning and disinfecting operations, wash basin and W.C. presence, establishment size, personal cleanliness of workers, existence of specific work clothing. Finally, the surface and internal temperatures of the samples were measured at the time of the collection with a digital probe.

2.2. Sample collection

At the slaughterhouse, samples were collected in the following way: a piece of meat of approximately 50 g was excised from the internal face of the thigh (no prolonged contact with the surroundings) and placed in a sterile plastic pouch. After collection, the pouches were placed at 0–10 °C in the slaughterhouse refrigerator, then transported to the laboratory (less than 24 h after collection) and stored in the freezer (–25 °C) until analysis.

At the retailers, after cutting up by the salesman, approximately 50 g of meat was sampled into a sterile plastic pouch then transported at 0–10 °C to the laboratory where they were frozen (–25 °C) until analysis.

2.3. *Salmonella* isolation and serotype determination

The samples were analyzed according to the NF V08-052 standard method (French Normalisation Association). Briefly, 25 g of each sample was placed in a sterile pouch sachet, 225 ml of

Buffered Peptone Water (Biorad/356-4684/Biorad/Marnes la coquette/France) were added, and the whole was homogenized on a Stomacher® (AES laboratoires/Stomacher 80/AES laboratoires/Combourg/France). Pre-enrichments were incubated at 37 °C for 16–20 h. Two milliliters and 0.1 ml of the pre-enrichment were then respectively transferred in 20 ml of selenite cystine broth (Biorad/356-4074/Biorad/Marnes la coquette/France) and 10 ml of Rappaport–Vassiliadis broth (Biorad/356-4324/Biorad/Marnes la coquette/France), and incubated for 18–24 h at 37 °C (selenite cystine) and at 42 °C (Rappaport Vassiliadis). Afterwards, one Hektoen Agar plate (Biorad/356-4284/Biorad/Marnes la coquette/France) per tube was inoculated and incubated at 37 °C for 18–24 h. Plates were then examined to identify *Salmonella* presence. Two presumptive colonies per sample were picked and grown on nutrient agar for purification, and then biochemically characterized using the Kligler–Hajna (Biorad/64844/Biorad/Marnes la coquette/France), urea–indole (Biorad/63713/Biorad/Marnes la coquette/France), Voges–Proskauer (Biorad/355-3911/Biorad/Marnes la coquette/France), and lysine decarboxylase tests (Biorad/355-3911/Biorad/Marnes la coquette/France). Agglutination tests were carried out on presumptive *Salmonella* strains by

the Enterobacteria National Reference Center, Dakar on the basis of O somatic antigens and phase 1 and phase 2 flagellar antigens agglutination with antisera (Bio-Rad, Marnes La Coquette, France) according to the White–Kauffman–Le Minor scheme.

2.4. Bacterial sensitivity tests to antibiotics

The sensitivity of strains to antibiotics were performed by the Enterobacteria National Reference Center, at the Institut Pasteur de Dakar, Senegal.

The following molecules were tested: beta-lactams (ampicillin, amoxicillin–clavulanic acid, ticarcillin, cefalotin, cefoxitin, cefotaxim, and ceftazidim), aminoglycosides (gentamicin, tobramycin, amikacin, and spectinomycin), quinolone antibiotics (nalidixic acid, ciprofloxacin, pefloxacin, and norfloxacin), tetracycline, chloramphenicol, nitrofurans, trimethoprim, sulphonamids (sulfamethoxazol) and the trimethoprim–sulfamethoxazol combination.

Inhibition zone diameters were read on the SIRSCAN antimicrobial susceptibility system (SIRSCAN 2000, Web version, I2A).

Table 1
Characteristics of beef retailers in Dakar, Senegal

	Modern butcher shops		Permanent markets		Districts' sales places		Itinerant retailers	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Number of inspected establishments	10	100	83	100	20	100	39	100
<i>Number of employees:</i>								
>10	3	30						
5 to 10	5	50						
<5	2	20	83	100	20	100	39	100
Wash basin	5	50	12	14.5	1	5	0	0
W.C.	6	60	65	78.3	4	20	0	0
Soap	2	20	0	0	0	0	0	0
Cleaning and disinfection operations	9	90	8	10	6	30	0	0
Clean cutting tools	7	70	8	10	6	30	0	0
<i>Covering materials of cutting tables</i>								
Resin	7	70			2	10		
Plastic			3	3.6	4	20		
Wood	3	30						
Wood and cardboard			80	96.4	14	70		
Cardboard							39	100
Specific work clothing	6	60	12	14.5	7	35	0	0
Good personal hygiene	3	30	7	8.5	1	5	0	0
<i>Meat transfer temperature</i>								
>10 °C	3	30	56	67	15	75	39	100
0–10 °C	7	70	25	30	5	25		
<0 °C			2	2				
<i>Storage temperature</i>								
>10 °C	4	40	80	96	14	70	39	100
0–10 °C	6	60	1	1.2	5	20		
<0 °C			2	2.4	1	10		
Average internal temperature of the sample (°C)	15.1		26.6		21.2		30.3	

Table 2
Salmonella prevalence in beef sampled at the slaughterhouse and retailers in Dakar, Senegal

Samples source	Number of samples		
	Analyzed	Positive	% [CI]
Slaughterhouse	236	101	42.8 [36.6–49.2]
Retail :	199	174	87.4 [82.3–91.5]
— Modern butcher shops	34	24	70.6 [53.8–84.0]
— Markets	83	82	98.8 [94.2–99.9]
— District retailers	43	29	67.4 [52.5–80.1]
— Itinerant retailers	39	39	100.0 [92.6–100.0]
Total	435	275	63.2 [58.6–67.7]

2.5. Statistical analysis

The Statcalc module of the Epi Info™ software was used to perform Ki2 tests on the results, with 10% accuracy and 5% risk.

3. Results

3.1. Characterization of the slaughterhouse samples

The information obtained at the slaughterhouse allowed us to characterize the animals from which the samples were collected. The carcasses sampled were distributed equally between intensive ($n=117$) and extensive rearing system ($n=119$). The animals coming from intensive breeding were significantly heavier than those from extensive breeding.

In Senegal, the intensive system is generally a final fattening stage for animals reared extensively. Nevertheless, some stockbreeders are developing practices close to those used in western developed countries.

The animals were 78% Peulh zebu (Gobras) ($n=183$), 20% Moors zebu ($n=47$), 2% Ndamas ($n=5$), and one animal was not identified. Bulls represented 83% ($n=197$) of the animals, cows 14% ($n=32$) and castrated animals 3% ($n=7$).

Most of the animals ($n=92$, 39%) came from the Zone Sylvo-Pastorale, an extensive cattle-breeding area in the north-east of Senegal while 22% (52) came from the Bambey–Djourbel zone, an intensive cattle-breeding area, east of Dakar. Finally, 11% of the animals ($n=25$) came from Mali.

About half of the carcasses ($n=110$, 47%) were of good shape, 11% of bad conformation ($n=27$), the remainder ($n=99$) of average conformation. This was visually judged by the veterinary of the slaughterhouse.

3.2. Characterization of the retailers' samples

The survey analysis enabled us to group the beef retailers (Table 1). Of the 4 types investigated, only modern butcher shops were large, usually employing more than 5 persons, other structures employed one to two people. Modern butcher shops were also the ones more frequently having a washbasin, cutting tables made of resin and specific work clothing. Most other establishments globally had poor equipment hygiene and poor hygiene practices. While retailers usually transported and stored the meat at room temperature, modern butcher shops frequently executed these steps at 0 to 10 °C.

3.3. Isolated *Salmonella* prevalence

Among the 435 samples analyzed, 63% (275) were positive for *Salmonella*. Of the 236 slaughterhouse samples, 101 (43%) were positive for *Salmonella*. At the retailers, 87% (174) of the samples were contaminated by one or more *Salmonella* (Table 2). Furthermore two different serotypes were isolated from 11 samples. In summary, 71% (24) of the modern butcher shops samples, 99% (82) of the market samples, 67% (29) of samples from district retailers and 100% (39) of the samples from itinerant retailers were positive for one or more *Salmonella* (Table 2). Samples positive for *Salmonella* were detected from 97% (150) of the retailers. Only one modern butcher shop and one district retailer provided samples free from *Salmonella*.

Table 3
Main *Salmonella* serotypes isolated from beef sampled at the slaughterhouse and from retailers in Dakar, Senegal

	Slaughterhouse	Retail				Retailers total	Total
		Modern butcher shops	Permanent markets	Districts retailers	Itinerant retailers		
Number of strains tested	96	19	72	20	27	138	234
<i>Abaetetuba</i>		2	2			4	4
<i>Brandenburg</i>	1	1	1	1		3	4
<i>Bredeney</i>	69	1	1			2	71
<i>Corvallis</i>		2	6	1	3	12	12
<i>Hindmarsh</i>	9						9
<i>Kentucky</i>	1		9			9	10
<i>Montevideo</i>			4			4	4
<i>Muenster</i>	3	6	8	3	1	18	21
<i>Offa</i>		1	1	1	1	4	4
<i>Oranienburg</i>	6				1	1	7
<i>Poona</i>		1	3			4	4
<i>Sendai</i>			5		1	6	6
<i>Waycross</i>		3	2	9	4	18	18
<i>Westphalia</i>					5	5	5

3.4. Slaughterhouse sample characteristics and *Salmonella* prevalence

No significant difference ($p=0.77$) between intensive and extensive stock rearing practices was found. Furthermore, neither the animals' area of origin ($p=0.35$), weight ($p=0.51$), age ($p=0.30$), sex ($p=0.18$), race ($p=0.50$), or carcass conformation ($p=0.29$) had any impact on the prevalence of *Salmonella*.

3.5. Retailers samples characteristics and *Salmonella* prevalence

Meat transfer at room temperature did not seem to increase the risk to find *Salmonella* in the samples ($p=0.02$); on the other hand, storage at room temperature did increase it ($p=0.008$). The complete data on establishment hygiene confirmed that it did affect the risk of detecting *Salmonella* in meat: the tools cleanliness ($p=0.00005$), cleaning and disinfecting ($p=0.00002$), coating of cutting tables ($p=0.008$), specific work clothing ($p=0.00003$) and standard of personal cleanliness ($p=0.002$) decreased the risk of *Salmonella* presence in meat.

Table 4
Resistance to antibiotics of *Salmonella* strains isolated from beef sampled at the slaughterhouse and retailers in Dakar, Senegal

Samples source	% of strains resistant to antibiotics					Total
	Slaughterhouse	Modern butcher shops	Markets	Retails places	Itinerant retailers	
Number of strains tested	99	20	76	23	29	247
AMC	0	0	0	0	0	0
AMX	0	0	0	0	0	0
AN	0	0	0	0	0	0
TMP+SSS	0	0	0	0	0	0
C	2.0	0	0	0	0	0.8
CAZ	0	0	0	0	0	0
CF	0	0	0	0	0	0
CIP	0	0	0	0	0	0
CTX	0	0	0	0	0	0
FOX	0	0	0	0	0	0
FT	36.7	65.0	64.5	78.3	69.0	62.4
GEN	0	0	0	0	0	0
NA	1.0	0	0	0	0	0.4
NOR	0	0	0	0	0	0
PEF	1.0	0	0	0	0	0.4
S	14.1	15.0	25.0	43.5	24.1	21.5
SPT	0	0	0	8.7	0	1.1
SSS	21.1	10.0	3.9	39.1	6.9	14.7
TE	0	0	1.3	0	0	0.4
TIC	0	0	0	0	0	0
TM	0	0	0	0	0	0
TMP	0	0	0	0	0	0

AMC: amoxicillin+clavulanic acid, AMX: amoxicillin, AN: amikacin, C: chloramphenicol, CAZ: ceftazidime, CF: cefalotin, CIP: ciprofloxacin, CTX: cefotaxim, FOX: cefoxitine, FT: nitrofurans, GEN: gentamicin, NA: nalidixic acid, NOR: norloxacin, PEF: pefloxacin, S: streptomycin, SPT: spectinomycin, SSS: sulfamethoxazole, TE: tetracyclin, TIC: ticarcillin, TM: tobramycin, TMP: trimethoprim.

Table 5

Resistance to antibiotics repartition for *Salmonella* isolated from bovine meats sampled at the slaughterhouse and retailers in Dakar, Senegal

Number of isolates	Slaughterhouse	Modern butcher shops	Markets	District retailers	Itinerant retailers
Tested	99	20	76	23	29
Sensitive	61	7	21	3	6
Mono-resistant	30	9	41	9	17
Di-Resistant	7	3	11	5	6
Tri-resistant	1	1	3	4	0
Quadri-resistant	0	0	0	2	0
Total	8	4	14	11	6
multiresistant					
%	8.0	20.0	18.6	47.8	20.7
multiresistant confidence interval	[3.8–14.7]	[6.7–41.5]	[11.0–28.7]	[28.3–67.9]	[8.8–38.2]

3.6. Serotypes distribution

Salmonella isolated from the 275 positive samples were of 51 different serotypes. The most common, 56% of the isolates, were *bredeney* ($n=71$), *muenster* ($n=21$), *waycross* ($n=18$), *corvallis* ($n=12$) and *kentucky* ($n=10$) (Table 3).

At the slaughterhouse, *S. bredeney* largely prevailed ($n=69$, 71% of the isolates), followed by *S. hindmarsh* ($n=9$, 9.7%) and by *S. orianenburg* ($n=6$, 6.45%). The Ki2 test did not give a significant difference in the frequency of serotype isolation depending on breeding practice (intensive/extensive), race, geographical origin or carcass conformation.

Concerning the samples from modern butcher shops, the 19 isolates were of 10 serotypes. *S. muenster* (6; 24.0%), *S. waycross* (3; 12.0%), *S. corvallis* and *S. abaeetuba* (2; 8%) represented around 50% of the isolates.

The 72 *Salmonella* isolates from markets meats were from 33 serotypes: *S. kentucky* (9; 11.1%), *S. muenster* (8; 9.9%), *S. corvallis* (6; 7.4%), *S. sendai* (5; 6.2%) and *S. montevideo* (4; 4.9%) prevailed.

In samples from district retailers, the 20 isolates belonged to 10 serotypes: *S. waycross* (9; 29.0%) and *S. muenster* (3; 9.7%) prevailed.

The 27 isolates from itinerant retailers meats were divided into 18 serotypes: *S. westphalia* (5; 13.5%), *S. waycross* (4; 10.8%), and *S. corvallis* (3; 8.1%) represented 42% of the isolates.

Some serotypes (*S. muenster*, *S. bredeney*, and *S. brandenburg*) were found both in slaughterhouse and in retailers' samples. *S. bredeney*, which prevailed at the slaughterhouse, appeared only rarely at retailers. Others serotypes (*S. corvallis*, *S. waycross*, *S. offa*) seemed retailer specific.

3.7. Antibiotic resistance

A high proportion of the strains was resistant to nitrofurans (111; 62.4%), and to a lesser extent to streptomycin (53; 21.5%) and to sulfamethoxazol (35; 14.7%) (Table 4). Two isolates were resistant to spectinomycin, two to chloramphenicol, one to tetracycline and one to pefloxacin. Similar rates of resistant strains

were observed in samples from modern butcher shops, markets and itinerant retailers. Strains isolated from slaughterhouse meat were less resistant to nitrofurans. Those from the district retailers' meats were proportionally more resistant: the difference was significant in comparison with slaughterhouse samples and samples from markets when comparing the places two to two. The proportion of multi-resistant strains in retailers' samples confirmed these data (Table 5).

3.8. Serotypes and resistance to antibiotics (Table 5)

On the 247 investigated isolates, 55 (22%) were not resistant, 149 (60%) were resistant to at least one and 43 (17.4%) were resistant to more than two antibiotics from different families. Table 6 summarizes the resistance patterns of the most frequent serotypes.

Among the more frequently found serotypes, *S. bredeney*, primarily found at the slaughter-house, comprised six different resistance types. *S. corvallis*, found in meat from all four of the retailers types, comprised only two different resistance profiles. The majority (7; 70%) of *S. kentucky* isolates was from the same market and comprised three resistance patterns.

Table 6
Resistance phenotypes and sources of the *Salmonella* strains most frequently found in beef in Dakar, Senegal

Serotype	Source	Number of <i>Salmonella</i> isolates			
		Tested	Resistant	Multiresistant	Antibiotic type
<i>Bredeney</i>	Slaughterhouse	70	28	7:	6 S, 2 FT, 10 SSS, 2 C
				6	FT S
				1	S SSS
<i>Corvallis</i>	Butcher shops	1			
	Markets	1	1	1	FT S
	Butcher shops	2	2	1	FT S
	Markets	6	3		FT
	Retail places	1	1		FT
<i>Kentucky</i>	Itinerant retailers	3	1		FT
	Slaughterhouse	1	1		FT
	Market	9	9	7:	FT
<i>Muenster</i>	Slaughterhouse	4	1		FT S SSS
				6	FT S
				1	FT S SSS
	Butcher shops	6	3	1	FT SSS
	Markets	8	7		FT
	Retail places	3	3	3:	FT S SSS
				1	FT SSS
<i>Waycross</i>	Itinerant retailers	1	1	2	FT
	Butcher shops	3	3	2:	FT
	Markets	2		1	FT S
				1	FT S SSS
				1	FT S SSS
	Retail places	9	8	4:	3 FT, 1S
				2	FT S SPT SSS
<i>Waycross</i>	Itinerant retailers	4	4	1	FT S
				2	FT S
					FT S

S. muenster was found in all district retailers' samples: the 22 identified isolates were from twelve different collection places and comprised four resistance profiles. *S. waycross* isolates were from samples collected in twelve different places and comprised five different patterns.

Six samples collected the same day from different retailers yielded eight isolates, all multi-resistant. Six were *S. waycross* (FT S, FT S SSS, FT S SPT SSS), one was *S. muenster* FT S SSS and one *S. vinhoady* FT S SSS. The last two had been isolated together with one *S. waycross* FT S SSS and one FT S SPT SSS respectively. Three of the retailers belonged to the same company and were supplied by the same vehicle so a common contamination source can be considered.

4. Discussion

4.1. Prevalence and distribution of *Salmonella* serotypes

High prevalence rates of *Salmonella* isolated from beef at the slaughterhouse (43%) and among retailers (90%) highlight a very strong contamination of the bovine meat chain in Dakar.

The samples were frozen prior to analysis, which could have affected the recovery of some strains and serotypes. However, the high *Salmonella* prevalence indicate that this effect was probably not major, although low levels of contamination may not have been identified after freezing.

In an Ethiopian slaughterhouse, *Salmonella* contaminated 5.6% of meat samples (Molla et al., 2003). For confirmation, the authors used Brilliant-Green and Macconkey agars. A Nigerian survey found 25% of *Salmonella* prevalence in beef samples at the slaughterhouse, and 15.6% at retail (Orji et al., 2005). In another survey conducted at a South-African slaughterhouse, 60% of the samples were positive (Nel et al., 2004). Nel et al. worked on 10 g of sample and used Rappaport–Vassiliadis broth (Oxoid) as single selective enrichment broth. In Australia, *Salmonella* was found in 0.1% of slaughterhouse beef samples; in that case surveys were carried out on surface samples collected using sponge collecting kits (Phillips et al., 2001). In Ireland, 7.6% of the carcasses were contaminated (McEvoy et al., 2003) using immunomagnetic capture from impregnated sponges. In a survey of meat marketed in Ethiopia, Ejeta et al. found that 15% of the samples were positive for *Salmonella* (Ejeta et al., 2004).

Differences in sample collection as well as the various techniques used in these different surveys can explain most of the important variations observed among prevalence. However, the rates found in Senegal are still particularly high. There is a noticeable correlation between better hygiene and a relatively lower prevalence.

It is worthy to note that more serotypes were isolated at retailers (47 serotypes) than at the slaughterhouse (11 serotypes). Some serotypes such as *bredey*, *kentucky*, *muenster*, and *offa* were found in both types of samples. *S. bredeney* was largely prevalent at the slaughterhouse, but was rarely found in retailers' samples. At the slaughterhouse the former serotype exhibited six profiles of resistance to antibiotics. The survey did not enable the serotypes found at the slaughterhouse to be linked to the slaughtered animals. It is therefore probable that strains were specific to the

slaughterhouse. *S. bredeney*, rare among retailers' samples, may have been masked by other serotypes, or was less specific to the meat or was less competitive than the serotypes found later on, although sample variation and cross-contamination in the slaughterhouse may have explained this variability. Indeed, in Ireland, *S. bredeney* was very common in retail meat (Duffy et al., 1999) and in poultry implicated in food poisoning (Moore et al., 2003). Other serotypes were retailer specific (*waycross*, *corvallis*, *muenster*, *sendai*). Some serotypes were present in all four retail types (*waycross*, *corvallis*, and *muenster*). Thus one possibility is that it could be present at slaughter without being detected or while being hidden by other dominant serotypes. On the other hand, other serotypes specific to some retail places could come from a contamination at a later date (*kentucky*, *abaetetuba*, *sendai*, *montevideo*, *altona*, and *westphalia*). Most of the *S. kentucky* was found in samples from the same market. Like *S. bredeney* in the slaughterhouse, it could be a serotype specific to this market place. The survey carried out among retailers highlighted poor hygiene conditions, regarding the temperature of storage, the equipment as well as the employees' personal hygiene. The cutting tables were seldom washed or disinfected before use. These benches could therefore be reservoirs from which *Salmonella* could spread to other equipment through flies or direct contact. Trades not respecting elementary hygiene rules (markets and itinerant salesmen), had the highest contamination rates. Two different serotypes were found in eleven samples. If more colonies have been picked by sample, we might have found more serotypes, masked by dominant ones. In this work however, isolated colonies were randomly selected among characteristic ones for confirmation. The bias is therefore weak, but a different competitiveness for each serotypes cannot be excluded. There were an interval of one year between the samples collection at the slaughterhouse and at retailers; we thus cannot exclude seasonal serotype variation. However, it is notable that some serotypes were found in equivalent proportions in 2003 and 2004. Sample collection was carried out several times in modern butcher shops as well as at districts retailers, whereas it was carried out only once at markets and itinerant vendors, these being more numerous. The representativeness is consequently not the same, so the results should be globally considered by trade type. An Ethiopian survey (Molla et al., 2003) carried out at the abattoir show *S. braenderup*, *S. dublin*, and *S. saintpaul* prevailed. A survey undertaken on meats in Ireland (Duffy et al., 1999) indicated the prevalence of the *bredeney*, *kentucky* and *enteritidis* serotypes. So this serotypes are common on meat even if economical and environmental conditions of Senegal and Ireland cannot be compared. Another survey carried out in the United States (Schlosse et al., 2000) detected *montevideo*, *typhimurium*, *muenster* and *kentucky* serotypes' on carcasses, and *montevideo*, *anatum*, *muenster* and *kentucky* in beef. The results from Senegal are unique, there is a large geographical variety, but the *S. bredeney*, *S. kentucky* and *S. muenster* serotypes were still rather generally found.

4.2. Antibiotic resistance

In this survey, no link between resistance to antibiotics and the serotypes was found. There is a high prevalence of resistant strains

but no strain was resistant to more than four antibiotics. Resistance to nitrofurans was widespread. Development of nitrofurantoin resistance can be the result of a wrong use of antibiotics in animal husbandry. They can be used as anti-coccidia drugs. In human medicine, they are used to treat urinary infections; therefore existence of *Salmonella* resistant to this type of antibiotic has a potentially negative impact on human health. Also the small number of multi-resistant strains indicate that the use of antibiotics in animal husbandry remains moderate. Currently, animal rearing with intensive antibiotic use appears to be emerging since multi-resistant *S. waycross* isolates were identified in samples collected at different retailers on the same day. The origin of the meat was not formally identified but three of the retailers were supplied from the same vehicle. Of the four retailer types, the samples from district retail places had the lowest *Salmonella* prevalence, but the highest proportion of multi-resistant strains. This high rate was amplified by collection of three samples on the same day from which five multi-resistant strains were isolated. These five strains excluded, the multi-resistant *Salmonella* rate is comparable with the other retail outlets.

In Ethiopia, Alemayehu et al. (2003) showed 52% of the *Salmonella* isolated at the slaughterhouse from beef were resistant to at least three antibiotics. In the United States, 84% of the *Salmonella* isolates from retail meats were resistant to at least one antibiotic, and 53% to at least three antibiotics. Eighteen isolates accounting for four serotypes contained integrons coding for resistances to the aminoglycosides, sulphonamides, trimethoprim and betalactams (White et al., 2001). In a survey of *S. muenchen*, isolated from pigs in the United States, the authors found that 75% of the isolates were resistant to seven antibiotics and that one human isolate was resistant to 10 antibiotics. Pulsed field analysis did not show the strains' clonality. In vitro, the strains were able to transfer their resistance plasmids to *E. coli* (Gebreyes and Thakur, 2005). *Salmonella* isolated from meat products in Ireland were 100% resistant to rifampicin, 92% resistant to tetracycline, 86.3% to oxytetracycline, 86.3% to sulfamethoxazole and 80.9% to streptomycin (Duffy et al., 1999).

By comparison, therefore, the strains isolated in Dakar showed a low incidence of resistance.

In Senegal, the mainly extensive cattle raising system, is not a factor in the appearance of highly multi-resistant strains. However a minority of farms seem to be changing their practices and producing more resistant strains. The antibiotic profiles in those cases are beginning to resemble those observed in modern western countries where antibiotics are widely used.

4.3. Human distribution of *Salmonella* serotypes

The Enterobacteria National Reference Center, located at the Institut Pasteur de Dakar, identified, serotyped and tested the sensitivity to antibiotics of strains sent in by various laboratories in the Dakar area. The results published in 2003 listed the prevalent serotypes, among human cases between 1999 and 2003. They were *S. enteritidis* ($n=196$, 20.5%), *S. typhi* ($n=114$, 11.9%) and *S. typhimurium* ($n=70$, 7.3%). Those three serotypes accounted for nearly 40% of the strains of human origin (Perrier-Gros-Claude and Dromigny, 2003). *S. kentucky* ($n=47$, 4.9%)

appeared in fourth place, *S. montevideo* ($n=15$, 1.5%) and *S. bredeney* ($n=14$, 1.4%) in seventh and eighth position of the most frequently occurring serotypes in human samples. They were also found in this survey although in much more important proportion. Resistance to ampicillin (19.1%), clavulanic acid–amoxicillin (11.2%), nalidixic acid (5.6%), cotrimoxazole (17.9%), chloramphenicol (17.1%) and tetracycline (31.4%) was observed. These profiles are clearly different from those of the strains isolated from our meat samples. Food of animal origin is usually recognized as a source of human *Salmonella* infection (Morris, 1996), and *Salmonella* found in animals are frequently isolated from patients (Ekperigin and Nagaraja, 1998). Nevertheless, in Dakar, the main serotypes isolated in analyzed meats were not found in human infections. Similar results have been found in Kenya (Kariuki et al., 2002). Moreover, we did not observe any similarity between resistance patterns of the strains isolated from food and those from humans. A Brazilian survey reports the same phenomenon for *Salmonella* isolated from chicken meat and from humans (Oliveira et al., 2005). If *S. kentucky*, *S. bredeney* and *S. montevideo* have been found in both humans and meat, we also demonstrated that *S. bredeney* was rarely found in retail meat. Therefore, the correlation appears to be weak. Data on antibiotic resistance of strains from humans not being available for all strains, it was not possible to draw comparisons for *S. kentucky* and *S. montevideo*. Finer molecular investigations are necessary to compare these serotypes.

Serotypes occurring more frequently in these food products may be less virulent than the less frequent ones (*enteritidis*, *typhimurium*) (Carraminana et al., 1996). In addition, the infective dose of serotypes causing illness is probably lower than those of other serotypes. As we have no data about the number of *Salmonella* present in samples, the risk analysis is therefore limited. High number of *Salmonella* in meat products could lead to increased exposure, resulting in relative immunity of consumers to some serotypes (Wick, 2004). Finally, traditional culinary practices (long cooking time of meat in private households) would destroy the *Salmonella*. Human infection would more commonly occur after cross contamination from raw meat to other food. It would be interesting to know whether women, who more frequently cook and handle food, are more often infected by *Salmonella*. Lastly, a high contamination rate increases the exposure of the food chain and consumers.

5. Conclusion

This survey's main results are: (i) a very high *Salmonella* prevalence in retail beef; (ii) contamination at the slaughterhouse is amplified by poor hygiene practices and secondary contamination from resident flora; (iii) a high rate of resistance to antibiotics but a low rate of multiresistance; (iv) the emergence of multi-resistant strains in retail beef; (v) the weak relation between the strains isolated from beef and humans; (vi) very first data about meat contamination by *Salmonella* in the sub-saharian area.

Further molecular investigations are necessary to characterize more accurately some strains and to examine their degree of clonality. It would also be interesting to determine the development capacities of some serotypes on the meat as well as their

competitiveness in comparison with others. Finally, virulence characteristics of strains isolated both from meat and human samples could be examined.

Though the circulation of *Salmonella* in the beef production chain in Dakar is very important, to date, *Salmonella* strain from this source seem to have little impact on human health. However, high prevalence of *Salmonella* and sporadic emergence of multiresistant strains in beef in Dakar highlight the importance of introducing regulations and some training about the antibiotic use in animal husbandary before meat becomes a major source of multi-resistant strains problematic for human health.

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References

- Alemayehu, D., Molla, B., Muckle, A., 2003. Prevalence and antimicrobial resistance pattern of *Salmonella* isolates from apparently healthy slaughtered cattle in Ethiopia. *Trop. Anim. Health Prod.* 35, 309–319.
- Cardinale, E., Colbachini, P., Perrier-Gros-Claude, J.D., Gassama, A., Aidara-Kane, A., 2001. Dual emergence in food and humans of a novel multiresistant serotype of *Salmonella* in Senegal: *Salmonella enterica* subsp. *enterica* serotype 35:c:1,2. *J. Clin. Microbiol.* 39, 2373–2374.
- Carraminana, J.J., Yangüela, J., Blanco, D., Rota, C., Agustin, A.I., Herrera, A., 1996. Potential virulence determinant of *Salmonella* serotypes from poultry and human source in Spain. *Vet. Microbiol.* 57, 375–383.
- Cissé, M.F.G.-D.A., Boye, C.S., Boubakari, Y., Sow, A.I., Mboup, S., Samb, A., 1991. Salmonellosis: evaluation of 606 strains isolated in Dakar. *Bull. Soc. Méd. Afr. Noire Lang. Fr.* 36, 71–75.
- Cissé, M.F.S.A.I., Dièye-Sarr, E., Boye, C.S., Gaye-Diallo, A., Diop, D., Mboup, S., Samb, A., 1993. Antibiotic sensitivity of *Salmonella* strains isolated in a pediatric population in Dakar. Research of beta-lactamase and plasmids. *Bull. Soc. Pathol. Exot.* 86, 43–47.
- Duffy, G., Cloak, O.M., O'Sullivan, M.G., Guillet, A., Sheridan, J.J., Blair, I.S., McDowell, D.A., 1999. The incidence and antibiotic resistance profiles of *Salmonella* spp. on Irish retail meat products. *Food Microbiol.* 16, 623–631.
- Ejeta, G., Molla, B., Alemayehu, D., Muckle, A., 2004. *Salmonella* serotypes isolated from minced meat beef, mutton and pork in Addis Ababa, Ethiopia. *Rev. Med. Vet., Toulouse* 155, 547–551.
- Ekperigin, H.E., Nagaraja, K.V., 1998. Microbial foodborne pathogens. *Vet. Clin. North Am., Food Anim. Pract.* 14, 17–29.
- Garin, B., Aidara, A., Spiegel, A., Arrive, P., Bastarud, A., Cartel, J.L., Aissa, R.B., Duval, P., Gay, M., Gherardi, C., Gouali, M., Karou, T.G., Krui, S.L., Soares, J. L., Mouffok, F., Ravaonindrina, N., Rasolofonirina, N., Pham, M.T., Wouafo, M., Catteau, M., Mathiot, C., Maucelere, P., Rocourt, J., 2002. Multicenter study of street foods in 13 towns on four continents by the food and environmental hygiene study group of the international network of Pasteur and associated institutes. *J. Food Protect.* 146–152.
- Gebreyes, W.A., Thakur, S., 2005. Multidrug-resistant *Salmonella enterica* serovar *muenchen* from pigs and humans and potential interserovar transfer of antimicrobial resistance. *Antimicrob. Agents Chemother.* 49, 503–511.

- Kariuki, S., Revathi, G., Gakuya, F., Yamo, V., Muyodi, J., Hart, C.A., 2002. Lack of clonal relationship between non-typhi *Salmonella* strain types from humans and those isolated from animals living in close contact. *FEMS Immunol. Med. Microbiol.* 33, 165–171.
- Lafaix, C.C.M., Denis, F., Diop Mar, I., 1979. Salmonellosis in Dakar: bacteriological, clinical, epidemiological and therapeutic aspects. Ten years records. *Med. Trop.: Rev. Corps Sante Colon.* 39, 39–379.
- McEvoy, J.M., Doherty, A.M., Sheridan, J.J., Blair, I.S., McDowell, D.A., 2003. The prevalence of *Salmonella* spp. in bovine faecal, rumen and carcass samples at a commercial abattoir. *J. Appl. Microbiol.* 94, 693–700.
- Molla, B., Alemayehu, D., Salah, W., 2003. Sources and distribution of *Salmonella* serotypes isolated from food animals, slaughterhouse personnel and retail meat product in Ethiopia : 1997–2002. *Ethiop. J. Health Dev.* 17, 63–70.
- Moore, J.E., Murray, L., Fanning, S., Cormican, M., Daly, M., Delappe, N., Morgan, B., Murphy, P.G., 2003. Comparison of phenotypic and genotypic characteristics of *Salmonella bredeney* associated with a poultry-related outbreak of gastroenteritis in northern Ireland. *J. Infect.* 47, 33–39.
- Morris, J.J.G., 1996. Current trends in human diseases associated with foods of animal origin. *J. Am. Vet. Med. Assoc.* 209, 2045–2047.
- Motarjemi, Y., Kaferstein, F., Moy, G., Miagishima, K., Myagawa, S., Reilly, A., 1995. Food technologies and public health, food safety issues, WHO/FNUF/FOS/95.12.
- Nel, S., Lues, J.F.R., Buys, E.M., Venter, P., 2004. Bacterial populations associated with meat from the deboning room of a high throughput red meat abattoir. *Meat Sci.* 66, 667–674.
- Oliveira, S.D., Flores, F.S., Santos, L.R., Brandelli, A., 2005. Antimicrobial resistance in *Salmonella enteridis* strains isolated from broiler carcasses, food, human and poultry-related samples. *Int. J. Food Microbiol.* 97, 297–305.
- Orji, M.U., Onuigbo, H.C., Mbata, T.I., 2005. Isolation of *Salmonella* from poultry droppings and other environmental sources in Awka, Nigeria. *Int. J. Infect. Dis.* 9, 86–89.
- Perrier-Gros-Claude, J.D., Dromigny, J.A., 2003. Rapport 2003 du Centre National Sénégalais des Entérobactéries. Institut Pasteur de Dakar, Dakar, p. 32.
- Phillips, D., Sumner, J., Alexander, J.F., Dutton, K.M., 2001. Microbiological quality of Australian Beef. *J. Food Protect.* 64, 692–696.
- Samuel, J.L., O'Boyle, D.A., Mathers, W.J., Frost, A.J., 1980. Distribution of *Salmonella* in the carcasses of normal cattle at slaughter. *Res. Vet. Sci.* 28, 368–372.
- Schlosse, W., Hogue, A., Ebel, E., Rose, B., Umholtz, R., Ferris, K., James, W., 2000. Analysis of *Salmonella* serotypes from selected carcasses and raw ground product sampled prior to implementation of the Pathogen Reduction; Hazard Analysis and Critical Control Point Final Rule in the US. *Int. J. Food Microbiol.* 58, 107–111.
- Threlfall, E.J., 2002. Antimicrobial drug resistance in *Salmonella*: problems and perspectives in food- and water-borne infections. *FEMS Microbiol. Rev.* 26, 141–148.
- White, D.G., Zhao, S., Sudler, R., Ayers, S., Friedman, S., Chen, S., McDermott, P.F., McDermott, S., Wagner, D.D., Meng, J., 2001. The isolation of antibiotic-resistant *Salmonella* from retail ground meat. *New Engl. J. Med.* 345, 1147–1154.
- Wick, M., 2004. Living in the danger zone : innate immunity to *Salmonella*. *Curr. Opin. Microbiol.* 7, 51–57.