OPEN ACCESS Freely available online

Journal of Tropical Diseases and Public Health

Research Article

Phenotypic and Genotypic Characterization of *Salmonella* Isolated from Asymptomatic Carriers in the Suburb of Dakar

Niang Aissatou Ahmet[1-3*#], Sambe Ba Bissoume[1#], Seck Abdoulaye[2,4], Diop Amadou[2,5], Fall Ndèye Khota[1], Wane Abdoul Aziz[1], Bercion Raymond[4], Ka Roughyatou[6,7] , Sow Ahmad Iyane[2,3], Gassama Sow Amy[1,8]

[1]Experimental Bacteriology Unit, Dakar Pasteur Institute, Senegal; [2]Cheikh Anta Diop University of Dakar, Senegal; [3]Bacteriology Laboratory, National University Hospital Center of Fann, Senegal; [4]Medical Biology Laboratory, Dakar Pasteur Institute, Senegal; [5]Biology Laboratory, Albert Royer Children's Hospital, Senegal; [6]UFR of Health Sciences, University of Thiès, Senegal; [7]National Public Health Laboratory, Thiès, Senegal; [8]Dakar Polytechnic Higher School, Senegal

[#]These authors contributed equally to this study and share first authorship.

ABSTRACT

Salmonellosis is a major public health problem, especially with the emergence of multi-resistant *Salmonella*. The healthy carrier, an important factor of dissemination of *Salmonella* in the environment ensures inter-human transmission, especially in children. In Senegal, studies on (carrying *Salmonella* are lacking. Thus, this work was (was conducted) to determine the (carrying rate of *Salmonella*), characterize phenotypically and genotypically *Salmonella* strains isolated from healthy carriers in Dakar. This is a prospective study of five community sites (Jeddah Thiaroye Kao, Guinaw Rail South, North Pikine, Pikine East Guinaw North Rail) between January 2013 and April 2014. Phenotypic and molecular analysis PCR detection simplex virulence genes and typing by Multi-Locus Sequence Typing were performed at the Experimental Bacteriology Unit of the Institut Pasteur in Dakar. Sixteen *Salmonella* of one thousand eight hundred and eighty-eight (1888) stool specimens were identified to be a carrier rate of 0.84% for a variety of serotypes (Brancaster, Chester, Give, Poona, Agona, Johannesburg, Istanbul, Enteritidis, Corvalis). If all the *Salmonella* strains were sensitive to beta-lactams, 25% were resistant to at least one antibiotic (nalidixic acid, trimethoprim-sulfamethoxazole and tetracycline). All isolates show all virulence genes (invA, orfL, Pipd, SpiC, Misl). Meanwhile, the SpvR virulence gene was detected in one isolate associated with *Serovar enteritidis*. This reflects the pathogenicity degree of *Salmonella* strains and therefore their ability to cause human disease. The Multi-Locus Sequence Typing (MLST) technique has revealed that phenotypically identical serotypes had no allelic variation. This suggests that these clones were widely distributed geographically and are probably outstanding in a wide range of hosts. Two new ST were found with Chester and Brancaster. Considering the characteristics of isolated *Salmonella* in this study and the impact of carriers on public health, monitoring of healthy carriers is needed.

Keywords: *Salmonella* carrying virulence resistance; Multi-Locus Sequence Typing (MLST); PCR (Polymerisation Chain Reaction); Dakar; Senegal

INTRODUCTION

Salmonella is one of the main causes of foodborne diseases in the world and presents a major public health issue with a considerable cost to many countries. *S. typhi* accounts for about 21, 7 million cases of symptomatic infections a year with 217,000 deaths worldwide [1]. The emergence and spread of multi-resistant *Salmonella* strains have become a concern for health authorities and the scientific community.

In Africa, while many studies have focused on *Salmonella*-related *Salmonella* (sick, contaminated food), little work has been done

Correspondence to: Niang Aissatou Ahmet, Experimental Bacteriology Unit, Dakar Pasteur Institute, BP 5055 Dakar Fann, Senegal, Tel: 00221 77 501 37 15; E-mail: niangaisatou@yahoo.fr

Received date: December 21, 2019; **Accepted date:** January 22, 2020; **Published date:** January 28, 2020

Citation: Ahmet NA, Bissoume SB, Abdoulaye S, Amadou D, Khota FN, Aziz WA, et al. (2020) Phenotypic and Genotypic Characterization of *Salmonella* Isolated from Asymptomatic Carriers in the Suburb of Dakar. J Trop Dis 8:350. doi:10.35248/2329-891X.20.8.346

on healthy carriers. This port is threatened by the human serious, economic-related salmonellosis. In addition, this asymptomatic carriage may promote the intermittent release of the source of a *Salmonella* inter-human contamination, which can be important especially in children [2].

In Senegal, the frequency of *Salmonella* carriers in humans is unknown although it is a factor favoring the spread of these bacteria in the community.

Indeed, they carry with *Salmonella* genetic elements in virulence and resistance determinants that may compromise the efficacy of the treatment of patients.

So, a multicentre study CISAS/EPSA (asymptomatic carriers of *Salmonella* study in Africa) on community and hospital survey has been set up in Senegal and in some sub-Saharan African countries whose objectives were the control and prevention of disease Therefore we conducted this work, whose general objective was to study the isolated *Salmonella* carriers on sites in the suburbs of Dakar.

Objectives of the study

- Determine the carrying rate of in the study population
- Characterize phenotypically and genotypically strains isolated from carriers

MATERIALS AND METHODS

This is a community study of healthy carriers of *Salmonella* from January 2013 to April 2014 on five selected sites in Pikine. Households were randomly selected from the study area the typhoid fever the monitoring program in Africa PSFTA (area covered by health care facilities that diagnose and treat febrile diseases). All asymptomatic individuals regardless of gender or age living in the study area and have given free and informed consent were included in the study. Two investigators with the Global Positioning System (GPS) went into different households selected for enrolment (Figure 1). The collected stool samples were analysed at the experimental bacteriology unit IPD.

Figure 1: Location of study sites.

Identification of *Salmonella*

Salmonella specimens were identified using the standard protocol on the basis of morphological, cultural, biochemical and antigenic characters in suspicious colonies obtained after culture. Mueller Kauffman was used for enrichment that favored the growth of *Salmonella* that may be present in small quantities. After, stools were plated on Xylose Lactose Desoxycholate (XLD) and hecktoen agar and incubated at 37°C for 24 hours. Suspected non lactose fermenter colonies were subjected to biochemical reactions using kliger-hajna (uniquement, urease indole, citrate simmons and confirmed by Analytical Profile Index 20 Enteric (API 20E) according to manufacturers' instructions (BioMerieux SA, REF 20 190). Serotyping was done using slide agglutination using *Salmonella* polyvalent and monovalent O and H antisera (Diagnostic Pasteur, Paris, France) according to the Kauffmann-White classification scheme.

Serotyping

It was done at the National Centre for Enterobacteriaceae of Pasteur Institute Biomedical Analysis Laboratory of the IPD, by slide agglutination using *Salmonella* polyvalent and monovalent O and H antisera (Diagnostic Pasteur, Paris, France) according to the Kauffmann-White classification scheme. The antigenic formula was determined according to the scheme Kauffman-White.

Antibiotic susceptibility testing

Antimicrobial resistance tests were performed by the disc diffusion method on Mueller Hinton agar according to the standards recommendation of the French Society of Microbiology (CA-SFM, 2010). Discs with the following antibiotics (Bio-Rad, France) were tested ampicillin, amoxicillin +acid clavulanic, ticarcillin, cefalotin, cefoxitin, cefuroxime, cefotaxime, imipenem, nalidixic acid, aztreonam, norfloxacin, ciprofloxacin, gentamycin, tetracycline, streptomycin, cotrimoxazol. Sixteen antimicrobial disks were used in the following conditions: amoxicillin, amoxicillin-clavulanic acid, ticarcillin, cephalothin, cefoxitin, cefotaxime, ceftazidime, nalidixic acid, Flumequin, norfloxacin, trimethoprim-sulfamethoxazole, tetracycline, chloramphenicol, gentamicin, Amikacin, ciprofloxacin.

DNA extraction

The genomic DNA of the isolated *Salmonella* was extracted by using the chemical method (Qiamp DNA Mini Kit).

Molecular detection of virulence genes

The detection of six virulence genes was performed on all *Salmonella* strains isolated by simplex PCR using specific primer pairs: invA1/invA2, spiC1/spiC2, orfL1/orfL2, pipD1/pipD2 and spvR1/spvR2 (Table 1). Control strains (4/74 ATCC *S. typhimurium* et SS44 *S. abortusovis*) were used to validate the results.

Table 1: Primers used to search for virulence genes.

Primers	Primer sequence (5'?3')	Size	Function	Hybridization temperature	Controls
InvA1	TGCCTACAAGCATGAAATGG	450 pb	Invasive Gene	58°C	
InvA2	AAACTGGACCACGGTGACAA				4/74 ATCC: *Salmonella typhimurium*
SopB-F	CGGACCGGCCAGCAACAAAACAAGAAGAAG	220 pb	SPI-1	55°C	
SopB-R	TAGTGATGCCCGTTATGCGTGAGTGTATT				
SpiC-1	CCTGGATAATGACTATSTGAT	301 pb	SPI-2	54°C	
SpiC-2	AGTTTATGGTGATTGCGTAT				
MisL1	GTCGGCGAATGCCGCGAATA	561 pb	SPI-3	60°C	
MisL2	GCGCTGTTAACGCTAATAGT				SS44: *S. abortusovis*
OrfL-F	GGAGTATCGATAAAGATGTT	332 pb	SPI-4	60°C	
OrfL-R	GCGCGTAACGTCAGAATCAA				
PipD-1	CGGCGATTCATGACTTTGAT	399 pb	SPI-5	56°C	
PipD-2	CGTTATCATTCGGATCGTAA				
SpvR-1	CCCCGGGAATTCGCTGCATAAGGTCAGAAGG	890 pb	Virulence plasmid	47°C	
SpvR-2	CCCCGGGATCCATGGATTTCTTGATTAATAAA				

Multi-locus sequence typing of *Salmonella*

Amplification of all genes was performed with a 50 µl reaction mixture of the following: 10 X Buffer, 1.5 mM $MgCl_2$, 2 mM DNTP, 12.5 forward primer, 12.5 µL Forward primer, 5 U/µl Taq Polymerase, sterile DNA, free water. PCR cycling conditions were 10 nm at 94°C followed by 32 cycles of 94°C for 1 nm, 55°C for 1 nm, 72°C for 1 nm with a final extension at 72°C for 5 nm. From each PCR, 2 µl aliquots were separated by 1% agarose gel electrophoresis with ethidium bromide staining and the bands obtained were visualized under the ultraviolet lamp (312 nm) using a gel documentation system (Gel Doc 2000, Biorad).

The sequencing of products PCR was carried out in the centre of Beckman Coulter Genomics (United Kingdom). Products of PCR were purified by the kit QIA quick freezing extraction of Qiagen then sequences by using the protocol big dye terminator on the automat sequencer ABI PRISM 3100 following the recommendations of the manufacturer (Perkin-Elmer Applied Biosystems, Ulis, France). Sequences obtained were analysed at the National Reference Centre Salmonellas (Institute Pasteur Paris). The sequences were subjected to the database for Multi-Locus Sequence Typing (MLST) and of the numbers of alleles (existing or new) were allotted to the studied sequences. The data entry and the analysis were carried out on the level of the Unit of Epidemiology of the Institute Pasteur of Dakar. The software R and the test of Chi^2 were used (Table 2).

Table 2: Primers used for the characterization of *Salmonella* by MLST.

Locci	Primer sequence (5'-3')	Size (pb)	Tm
thrA	F: GTCACGGTGATCGATCCGGT R: CACGATATTGATATTAGCCCG	852	55 °C
pure	F1: GACACCTCAAAAGCAGCGT' R2: AGACGGCGATACCCAGCGG	510	
sucA	F1: CGCGCTCAAACAGACCTAC R1: GACGTGGAAAATCGGCGCC	643	
hisD	F1: GAAACGTTCCATTCCGCGC R1: GCGGATTCCGGCGACCAG	894	
aroC	F: CCTGGCACCTCGCGCTATAC	826	

	R: CCACACACGGATCGTGGCG	
hemD	F1: GAAGCGTTAGTGAGCCGTCT GCG R: ATCAGCGACCTTAATATCTTG CCA	666
dnaN	F: ATGAAATTTACCGTTGAACGT GA R: AATTTCTCATTCGAGAGGATT GC	833

F: Forward; R: Reverse; Tm: Hybridization temperature

RESULTS

Study population

On the whole, google earth made it possible to select 46510 structures made up of households; of the retail outlet (shops, etc.), 592 households were selected randomly and included in the study. With the device of geographical location, only 381 concessions were visited and 2268 subjects were included.

During the study period, one thousand eight hundred and eighty-eight (1888) agreed to give of their stools whose majority was obtained from DTK (669/1888) mean 35% followed by PW (381/1888) 20%, PE (262/1888) 13, 9%; GRN (257/1888) 13.6%; PN (195/1888)10.3% and from GRS (124/1888) 6.6% (Figure 2).

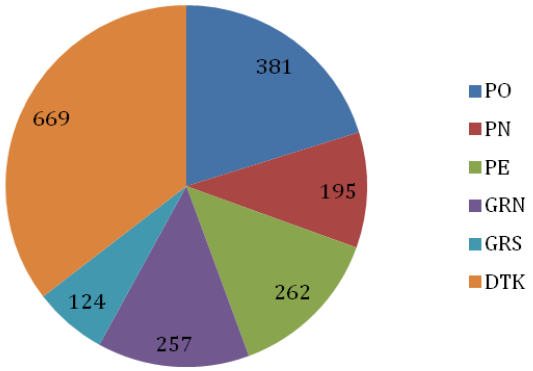

Figure 2: Distribution of samples by sites.

The distribution of the population according to sex showed that the female subjects were in majority: 1265 women against 718.

Salmonella carrier

Sixteen (16) non typhi stocks of *Salmonella* were identified on the one thousand eight hundred and eighty-eight (1888) samples of saddles (16/1888) which is to say a rate of bearing of 0.84% (p-value=2.2 E-16<0.05%). Eleven (11) strains were identified from women. The distribution of *Salmonella* was different according to the sites (Table 3).

Table 3: *Salmonella* distribution by study site.

Site	Number of stools collected	Number of isolated *Salmonella*
DTK	669	5
PW	381	4
PE	262	3
PN	195	4
GRN	257	0
GRS	124	0

Distribution of serovars

Nine (9) serovars were identified from 16 isolated *Salmonella* of which most dominant were represented by *S. Corvalis* (n=5); follow-up of the serotypes: Give (n=2), Johannesburg (n=2), Chester (n=2), Poona (n=1), Agona (n=1), Brancaster (n=1), Enteridis (n=1), Istanbul (n=1). The serotype Enteritidis was only found in one patient in Pikine West (PW) site while the *S. corvalis* serovar was the only isolated serotype in North Pikine (Table 4).

Table 4: Distribution of *Serovars* in the different study sites.

Serotypes		Isolation sites			
Name	**Antigenic formulas**	**DTK**	**PE**	**PW**	**PN**
Poona	13, 22: z: 1,6	-	-	1	-
Agona	4: f, g, s:-	-	-	1	-
Brancaster	4: z29:-	-	-	1	-
Enteritidis	9,12: g, m:-	-	-	1	-
Give	10: l, v: 1,7	-	2	-	-
Johanesburg	40: b: e, n, x	2	-	-	-
Istambul	8: z10: e, n, x	1	-	-	-
Chester	4: e, h: e, n, x	1	1	-	-
Corvalis	8: z4 z23:-	1	-	-	4

Susceptibility antibiotics

The analysis of the susceptibility of the *Salmonella* to antibiotics showed that four (25%) strains were resistant to at least an antibiotic. Thus, three profiles of resistance were observed: TER, NalR, Nal RSXTRTER;

These resistances concerned different *Serovars*. Indeed, resistance was observed with the tetracycline 19% (n=3) of strains (Chester, Brancaster, and Istanbul); with the acid nalidixic (n=3) (19%): Chester (2), Istanbul (1) and with association sulfaméthoxazole-triméthoprime (n=2) (13%): Chester and Istanbul.

Two Chester serotypes isolated at different sites had a different resistance profile to antibiotics. While Chester serotype isolated in East Pikine was resistant to nalidixic acid (NalR), that of Jedda Thiaroye Kao was resistant to nalidixic acid, tetracycline, and sulfamethoxazole-trimethoprim NalRSXTRTER .

All serotypes isolated in our study harbored almost all the required genes of virulence (p-value=2.2 e-16<0.05) (spiC, invA, spvR, pipD, orfL misL and sopB)) with exception of the spvR gene which found only in the Enteritidis serotype isolated at Pikine Nord (Figure 3).

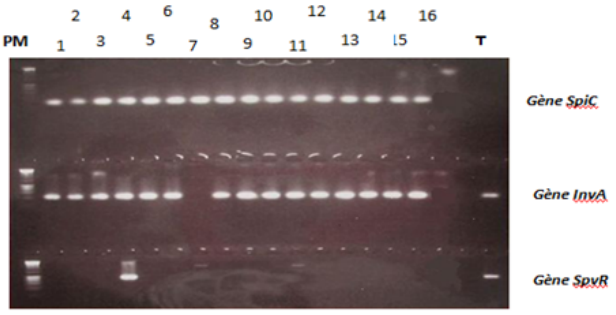

Figure 3: PCR result of genes: SpiC, Inv A, SpvR. PM: Molecular weight; T: Controls=474 ATCC-*S. typhimurium*.

Distribution of serovars

Nine (9) serovars were identified from 16 isolated *Salmonella* of which most dominant were represented by *S. Corvalis* (n=5); follow-up of the serotypes: Give (n=2), Johannesburg (n=2), Chester (n=2), Poona (n=1), Agona (n=1), Brancaster (n=1), Enteridis (n=1), Istanbul (n=1). The serotype Enteritidis was only found in one patient in Pikine West (PW) site while the *S. corvalis* serovar was the only isolated serotype in North Pikine (Table 5).

Table 5: Distribution the different study sites.

Serotypes		Isolation sites			
Name	**Antigenic formulas**	**DTK**	**PE**	**PW**	**PN**
Poona	13, 22: z: 1, 6	-	-	1	-
Agona	4: f, g, s:-	-	-	1	-
Brancaster	4: z 29:-	-	-	1	-
Enteritidis	9, 12: g, m:-	-	-	1	-
Give	10: l, v: 1, 7	-	2	-	-
Johanesburg	40: b: e, n, x	2	-	-	-
Istambul	8: z 10: e, n, x	1	-	-	-
Chester	4: e, h: e, n, x	1	1	-	-
Corvalis	8: z4 z23:-	1	-	-	4

MLST results

Each serotype corresponded to an ST. Of the 16 strains divided into 9 serotypes, nine Standard Sequences (ST) were identified

including two new STs discovered on Chester (n=2) and Brancaster serotypes. The two Chester serotypes that had a different resistance pattern had the same allelic profile (Table 6).

Table 6: MLST results of sixteen (16) isolated *Salmonella* serotypes in Portage.

	Profile of alleles							
Serotypes	*Aroc*	*dna*	*hemD*	*his D*	*purE*	*sucA*	*thrA*	*ST*
Johanesburg	13	109	49	157	12	13	4	515
Johanesburg	13	109	49	157	12	13	4	515
Corvalis	39	338	93	429	36	343	48	1357
Corvalis	39	338	93	429	36	343	48	1357
Corvalis	39	338	93	429	36	343	48	1357
Corvalis	39	338	93	429	36	343	48	1357
Corvalis	39	338	93	429	36	343	48	1357
Poona	22	104	25	113	12	83	4	308
Give	13	41	16	42	40	71	4	516
Give	84	11	16	42	40	71	4	516
Brancaster	-	-	10	36	88	108	36	New
Chester	11	10	25	42	337	13	4	New
Chester	11	10	25	42	337	13	4	New
Enteritidis	5	2	3	7	6	6	11	11
Istanbul	2	5	6	7	5	7	12	33
Agona	2	5	6	7	5	7	12	13

DISCUSSION

This study is the first in Senegal to characterize *Salmonella* isolated from asymptomatic human carriers. The feasibility of this study involved the intervention of different actors to carry out field and laboratory work. The precarious situations (floods, insalubrity) of most sites did not facilitate access to concessions for investigators who came to collect stool samples.

Moreover, in the field, obtaining the consent of the participants was difficult despite the efforts to raise awareness. However, the people included in the study benefited from free medical care (consultation, prescription, analyzes). The use of the GPS tool was necessary to identify households included in the study.

The sites were characterized by promiscuity and unsanitary conditions favored by floods thus constituting a favorable environment for the development of infectious diseases.

This study was performed on one thousand eight hundred and eighty-eight (1888) stool samples. The *Salmonella* carriage rate found in our study population was 0.84% (16/1888). The Chi^2 test used showed that the prevalence of carriage obtained was significant (p-value=2.2 e-16<0.05). This rate was relatively lower than those found in similar studies conducted long ago in African countries. In Senegal, a study of children in rural areas, found a carrying rate of 4.14% while in Côte d'Ivoire, a rate of 2.6% in school-aged children. The low rate observed in our study population could be explained by a weak circulation of the bacteria in the study area or by the intermittent excretion of these organisms, which is why successive collections would be interesting to detect more of carriers.

This carrier has been observed especially in women (p-value=0.672) because they are a potential source of *Salmonella* spread in their environment during their many activities (cooking, newborn sleeping, breastfeeding, etc.).

The story of Mary Mallon cook, better known as Mary Typhoid, carrier of *Salmonella* that unconsciously contaminates her customers, as she travels is a perfect illustration of the danger that can represent the carrying in the spread of the germ in the environment [3].

Nine *Salmonella* serotypes were isolated in the carrier in the suburbs of Dakar and the Corvallis serotype was predominant (n=5). Most of the serotypes isolated in our study are ubiquitous and were found in previous studies, particularly in the poultry sector and in cases of food poisoning [4].

The serotypes, Chester, Brancaster, Agona, Poona, were isolated in poultry samples but also in human pathology in Senegal [5,6]. Serotype Enteritidis, a major pathogen in public health, has been isolated from a single carrier. The study of antibiotic susceptibility of isolated strains showed no resistance to beta-lactamases and aminoglycosides. However, four (Brancaster, Istanbul, Chester (n=2)) of 16 or 25% showed resistance to tetracycline, sulfamethoxazole-trimethoprim and quinolone.

The emergence and diffusion of multi-resistant bacteria to antibiotics is a complex and worrying phenomenon, which can lead to great difficulties of care for the patients, with situations of therapeutic impasse [7].

This, especially since the inefficient antibiotics namely tetracyclines, sulfonamides and trimethoprim-sulfamethoxazole combination are the most used in Senegal to treat childhood diarrhea [8].

Salmonella resistance to these molecules could be promoted by their illicit sale, their uncontrolled use increasing the selection pressure that contributes to the emergence of resistant bacteria. These bacteria can then lead to a serious infection or contaminate other patients. Thus, tetracycline widely used in the world mainly because of its broad spectrum of action, its low toxicity but also its accessibility, is widely implicated in cases of *Salmonella* resistance.

The genetic determinants of resistance carried either by the chromosome or by transferable mobile elements (plasmids, transposons, integrons), can be transmitted to other bacteria of the same or different species and thus induce an emergence and

propagation of the bacterial resistance [9]. Thus, the state of carriage characterized by intermittent excretion of *Salmonella* could pose a real threat to the community as it spreads into the environment and spreads to other subjects. The observed resistance involved four different serotypes, including Istanbul, which was multiresistant against nalidixic acid, tetracycline, and cotrimoxazole; the frequent resistance of the Istanbul serotype has been reported in a previous study [10]. The emergence of *Salmonella* resistance to quinolones is a real public health problem; this is all the more worrying since the results from our study concerned isolated strains in patients without any clinical manifestation and could, therefore, be a source of contamination of their environment [11].

Most of the Chester serotypes found in animals were isolated in our study at two different sites and had a different resistance profile. This could be explained by the acquisition of resistance genes.

However, despite the good sensitivity of the strains to beta-lactams, they harbored almost all the desired virulence genes (invA, spiC, pipD, misL, orfL) contained respectively in SPI-1, SPI-2, SPI-5, SPI-3. These virulence genes located on patches of pathogenicity play a crucial role in the pathogenesis of *Salmonella enterica* infections and often result from a horizontal transfer between bacteria [12]. Despite their significant degree of pathogenicity, these strains remain in porting.

In addition, the factors, as well as the mechanisms implied in the asymptomatic bearing, are extremely badly known. Nevertheless, certain factors like the reduction in the gene expression of virulence or the expression of bacterial factors specific to the bearing could be accused [13]. These genes encoded by pathogenicity islands play an essential role in the different stages of *Salmonella* pathogenesis. The InvA gene is responsible for the virulence of *Salmonella*, in particular, the invasion of host cells by bacteria. All strains carrying this gene could potentially invade epithelial cells and therefore be virulent. On the other hand, this gene is a useful marker for molecular detection in *Salmonella* diagnostics [14,15]. Only serotype *Enteritidis* carried the gene (spvR).

The SPV operon is located in virulence plasmids found in specific serotypes including *Enteritidis* [14]. This gene was not found on strains of *S. enteritidis* isolated from various sources in southern Brazil [16]. However, the spvR gene was found on strains of *Salmonella nitra* of animal origin [17]. MLST is considered a method of choice for the characterization of a large number of pathogens.

This molecular typing technique based on the sequencing of household genes makes it possible to determine the genetic similarity within the serotypes. In our study, this technique showed that phenotypically identical serotypes did not show any allelic variation, that is, belonged to the same subtypes. This is the case of the *Corvalis* serotype (ST 1357) mainly isolated in the same site of North Pikine with the same phenotypic and genotypic characters. This suggests possible human-to-human contamination. In addition, another possible means of transmission via food could be incriminated following the isolation, in two different sites of two genotypically identical chester serotypes and with a different resistance profile, our results are compatible with those from earlier studies using the same MLST technique and not allowing a distinction to be made between closely related strains within the same serotype [18]. Furthermore, it has been reported that this same technique has a high discriminating power between the various *Salmonella* isolates. Thus, the study by Dione MM [10] made it possible to distinguish, by this same technique, phenotypically identical serotypes from Kentucky but with different standard sequences (ST): ST 198 and ST 832. Most *Salmonella* subtypes isolated from our study have already been described in previous studies [10,19] on *Salmonella* strains of various origins (animal, human and food). *S. agona* ST13 found in our human carriage study has also been described in the human carriers in Germany [19] and in chickens in Southern Senegal [10].

ST33 found in the Istanbul serotype was characterized by multidrug resistance (Nal-SXT-TE), which was similar to ST 33 found in a previous study [10] from chickens was much more resistant (more than five antibiotics). This shows that the emergence of multidrug-resistant *Salmonella* is a real phenomenon. However, the circulation of these virulent and multidrug-resistant strains is a public health problem because of their possible dissemination in the environment that may impact the effectiveness of therapeutic management. Indeed, the genetic supports of virulence and resistance can be transmitted to other diseases and to the former resistance and/or virulence

Given the circulation of these *Salmonella* in different sectors (human and food), it would be interesting to investigate the origin of strains isolated in portage in this study and to determine the molecular basis of antibiotic resistance.

However, this same ST33 subtype was found with the Hadar serotype from various sources in Europe [10]. In our study, the use of MLST showed that phenotypically identical *Salmonella* serotypes isolated in the same zone showed no genetic difference, means they belong to the same standard sequences. This suggests that these clones have widely distributed geographically and are likely circulating in a wide range of hosts.

However, in comparison with other molecular typing techniques, later studies have shown that MLST may be a good molecular epidemiological option for distinguishing isolates genetically indistinguishable by the PFGE technique. This low discriminating power has also been reported by other authors [20].

CONCLUSION

Resistance to these observed molecules makes even more complex the impact of the carry phenomenon for the population because of the possibility of diffusion of resistance genes and even *Salmonella* virulence genes, the latter being present in all isolates included in the study.

Some recommendations seem important to us, given the results obtained

- Survey of healthy carriers to avoid the spread of these strains harboring virulence and resistance genes

- Molecular surveillance of strains in the carriage and in the pathogenic situations

AUTHORS' CONTRIBUTION

This work was carried out as part of a project to monitor typhoid fever in Sub-Saharan Africa. We thank all the actors who participated in the success of this work.

REFERENCES

1. Crump JA, Luby SP, Mintz ED. The global burden of typhoid fever. Bull World Health Organ. 2004;82:346-353.
2. Gopinath S, Carden S, Monack D. Shedding light on Salmonella carriers. Trends Microbiol. 2012;20:320-327.
3. Roumagnac P, Weill FX, Dolecek C, Baker S, Brisse S, Tran Chin N, et al. Evolutionary history of Salmonella typhi. Science. 2006;24:1301-1304.
4. Bada-Alambedji R, Fofana A, Seydi M, Akakpo AJ. Antimicrobial resistance of Salmonella isolated from poultry carcasses in Dakar (Senegal). Braz J Microbiol. 2006;37:510-515.
5. Combari A. Level of contamination by antibiotic-resistant Salmonella from broiler farms in the peri-urban area of Dakar. EISM. 2014;57.
6. Dione MM, Ieven M, Garin B, Marcotty T, Geerts S. Prevalence and antimicrobial resistance of Salmonella isolated from broiler farms, chicken carcasses, and street-vended restaurants in Casamance, Senegal. J Food Prot. 2009;72:2423-2427.
7. Fofana A. Study of the antibiotic resistance of strains of Salmonella and Escherichia coli isolated from broiler meat in Senegal. DEA thesis of animal productions. 2004.
8. Toko MA. Assessment of the resistance level of Salmonella spp of avian origin to tetracycline and to sulfamethoxazole. PhD thesis, faculty of Medicine, Pharmacy and Odontostomatology of Dakar, Senegal. 2010. beep.ird.fr/collect/eismv/index/assoc/TD10-8.dir/TD10-8.pdf.
9. Beceiro A, Tomás M, Bou G. Antimicrobial resistance and virulence: a successful or deleterious association in the bacterial world? Clin Microbiol Rev. 2013;26:185-230.
10. Dione MM, Geerts S, Antonio M. Characterisation of novel strains of multiply antibiotic resistant salmonella recovered from poultry in Southern Senegal. J Infect Dev Ctries. 2012;6:436-442.
11. Roumagnac P, Weill FX, Dolecek C, Baker S, Brisse S, Chinh NT, et al. Evolutionary history of Salmonella typhi. Science. 2006;314:1301-1304.
12. Hensel M. Evolution of pathogenicity islands of Salmonella enterica. Int J Med Microbiol. 2004;294:95-102.
13. Amy Mt, Velge P, Senocq D, Bottreau E, Mompart F, Virlogeux-Payant I. Identification of a new Salmonella enterica Serovar enteritidis locus involved in cell invasion and in the colonisation of chicks. Res Microbiol. 2004;155:543-552.
14. Amini K, Salehi TZ, Nikbakht G, Ranjbar R, Amini J, Ashrafganjooei SB. Molecular detection of invA and SPV virulence genes in Salmonella enteritidis isolated from human and animals in Iran. Afr J Microbiol Res. 2010;4:2202-2210.
15. Oladapo OO, Kwaga JKP, Dzikwi AA, Junaid, K. Detection of inv A virulence gene by polymerase chain reaction (PCR) in Salmonella spp. isolated from captive wildlife. Bio-Genetics J. 2013;1:12-14.
16. Oliveira SD, et al. Detection of virulence genes in Salmonella enteritidis isolated from different sources. Braz J Microbiol. 2003;34:123-124.
17. Andrianony SA. Molecular characterization of Salmonella strains isolated from broiler farms in the peri-urban area of Dakar (Senegal), master thesis in human food quality. EISMV. beep.ird.fr/collect/eismv/index/assoc/TD14-5.dir/TD14-5.pdf.
18. Ross IL, Heuzenroeder MW. Discrimination within phenotypically closely related definitive types of Salmonella enterica Serovar typhimurium by the multiple amplification of phage locus typing technique. J Clin Microbiol. 2004;43:1604-1611.
19. Sangal V. Multilocus Sequence typing analyses of Salmonella enterica subspecies enterica: population structure, asymptomatic carriage and host association. 2008:1-114.
20. Stepan RM, Sherwood JS, Petermann SR, Logue CM. Molecular and comparative analysis of Salmonella enterica senftenberg from humans and animals using PFGE, MLST and NARMS. BMC Microbiol. 2011;11:153.