Original Article

Antimicrobial resistance and virulence genes of non-typhoidal *Salmonella* isolates in The Gambia and Senegal

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Abstract

Introduction: The prevalence of virulence genes in non-typhoidal *Salmonella* (NTS) and its association with commonly used antibiotics in West Africa is unknown.

Methodology: We tested 185 NTS isolates from children, animals, and food products for the presence of twelve virulence genes by PCR. Ten of the virulence genes tested belonged to the five *Salmonella* pathogenicity islands implicated in its pathogenesis.

Results: Ten of twelve virulence genes except *sopE* and *pefA* were present in at least 70% of the isolates tested; *sopE* and *pefA* were observed in 33% and 44% of the isolates, respectively. The most prevalent gene was *invA* (99.5%), which is an invasion gene conserved within the *Salmonella enterica*. *pipD* and *sopB* genes, which were associated with serovar Enteritidis, were detected in 92.4% and 94.1% of isolates respectively. *S.* Istanbul and *S.* Javiana, which were isolated from chicken-serving restaurants, carried all the virulence genes of the five pathogenicity islands. There was significant association between *sopB*, *sitC*, *orfLC*, *pipD* and *pefA* virulence genes and resistance to commonly used antibiotics in Senegal and The Gambia, namely amoxicillin, ticarcillin, trimethoprim plus sulfamethoxazole, tetracycline, trimethoprim, spectinomycin, streptomycin, sulfonamides and nitrofurantoin.

Conclusions: This study shows that virulence genes are present in NTS strains isolated from various sources. The significant association between some virulence genes and antibiotic resistance may have important implications with regard to the spread and persistence of resistance and virulence genes in *Salmonella* and to the prudent use of antimicrobial agents in humans and animals in West Africa.

Key words: non-typhoidal *Salmonella*; virulence genes; children; animals; West Africa

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Introduction

In sub-Saharan Africa including The Gambia and Senegal, non-typhoidal *Salmonella* (NTS) are a major cause of invasive disease in infants, young children, and HIV-infected adults and are associated with substantial mortality. For example, a retrospective study in Senegal revealed a prevalence of NTS in patients with HIV to be between 0.15% and 2.13% during 1996 to 2005. In addition, *Salmonella* serovar Enteritidis and *Salmonella* serovar Typhimurium were the most common serotypes [1,2]. In rural Gambia, NTS represents the second most common blood culture isolate after *Streptococcus pneumoniae* in children with invasive bacterial disease [3]. However, data on the prevalence of the animal origin of NTS in sub-Saharan Africa is limited. In Senegal, NTS infections are known to occur in semi-industrial poultry farms and contaminations were found at various levels of the poultry supply chain (retail chicken and street restaurants): 32% at sale place, 28.6% at farm level, and 20.1% at consumption level (street restaurant) [4-6].

The ability of NTS to cause invasive disease is attributed to arrays of virulence genes defined in the *Salmonella* pathogenicity islands (SPIs) [7]. There are at least 60 genes associated with SPIs [8] and the majority of these determinants are located on the chromosome or on large virulence-associated plasmids [9,10]. Five major SPIs are well characterized in *S.* Typhimurium and *S.* Typhi, namely, SPI1, SPI2, SPI3, SPI4 and SPI5 [8, 11], and these SPIs are the basis of pathogenicity in the host.

The emergence and spread of antimicrobial resistance among bacterial species including

Salmonella has become a public health threat. Antibiotic resistance, especially to the most commonly used antimicrobials in humans and in animal production systems, is of critical concern in African countries where multidrug-resistance NTS strains are among the most frequent causes of bacteraemia in children. The fact that genetic determinants for both antibiotic resistance and virulence genes could be harboured by the same transferable element [12] implies that there is an association between antibiotic resistance and virulence. Such association could have an impact on the spread of resistance clones of *Salmonella.* Consequently, assessing the presence of virulence genes as well as the antibiotic resistance status in *Salmonella* serovars would be useful to better understand its pathogenicity. Our study aimed to investigate the presence of twelve virulence genes in *Salmonella* serotypes and their association with antibiotic resistance.

Methodology

Sampling procedure and bacterial isolates

NTS isolates from Senegal were recovered from 57 broiler farms that were randomly selected from a total of 70 farms producing chickens all year round in urban and peri-urban settings. From one flock of each farm, five fresh fecal samples were collected in the chicken coop and pooled. Five chicken carcasses were also bought after slaughtering from each of the 57 farms or sale points, for a total of 285 carcasses. Skin (from the neck) and meat cuts were prepared under aseptic conditions. In addition, forty-two street restaurants that regularly serve chicken dishes were randomly chosen from a group of 50 restaurants in the same area. From each of these 42 restaurants, one pooled sample of three whole servings of chicken meat was collected. The collected samples (feces, skin, muscle, and chicken servings) were put into sterile plastic bags and stored in cool boxes during transportation to the microbiological laboratory where analysis was performed within 24hours.

In The Gambia, NTS isolates were obtained through an active population-based case-control surveillance study designated to determine the aetiology and epidemiology of enteric infections in Gambian children less than five years of age as part of the Gates Enteric Multicentre Study (GEMS). Children under five years of age who presented with severe diarrhoea (i.e., diarrhoea with dehydration, dysentery, or requiring hospitalization) within three days of onset were eligible to participate. For each enrolled child with diarrhoea, one healthy control child without diarrhoea was randomly selected from the community in which the case resided, matching the case by age, gender, and time of presentation. After receiving informed consent from the parent/guardian of each case and control, a single fresh, whole stool specimen was collected from cases and controls and cultured to detect *Salmonella*. The latter was isolated in the feces of 14 children (eight cases and six controls) enrolled in this study. The enrolled children were traced back to their family compounds and anal swabs were collected from apparently healthy animals (chicken, sheep and goats) residing in the same household within a week of isolating strains of NTS from humans. Five animals per species (chicken, sheep and goats) per compound were enrolled in the study, resulting in a total of 210 domestic animals (chicken, sheep and goats) or 70 per species. Thirty-five strains of NTS were isolated from the feces of 210 contact animals.

Salmonella isolates were characterised using standard protocols [13]. Stool specimens were transported in buffered glycerol saline (BGS) to the laboratory and processed within six hours of collection. Stools were plated on Xylose Lactose Desoxycholate (XLD) and MacConkey (MAC) agar and incubated at 36°C for 24 hours. Suspected non lactose fermenter colonies were subjected to biochemical reactions using Analytical Profile Index 20 Enteric (API 20E) according to manufactures' instructions (BioMerieux SA, REF 20 100/20 160). Serotyping was done at the Pasteur Institute Laboratory (Dakar, Senegal) by slide agglutination using *Salmonella* polyvalent and monovalent O and H antisera (Diagnostic Pasteur, Paris, France) according to the Kauffmann-White classification scheme [14]. Due to the large number of *Salmonella* serovar Brancaster, random selection using the simple random sampling procedure with Excel (Microsoft, Reading, UK) was performed. In the end, 40/148 isolates were considered for the study. The final analysis was conducted on 150 isolates from Senegal and 35 isolates from The Gambia, making a total of 185 isolates.

Antibiotic susceptibility testing

Antimicrobial resistance tests were performed on Muller-Hinton agar (Oxoid, Ogdensburg, NY, USA) using the agar diffusion method with antimicrobial disks (Bio-Rad, Marne-La-Coquette, France) according to the guidelines of the Antibiogram Committee of the French Society for Microbiology

Table 1. Virulence genes and PCR primers used to screen *Salmonella* isolates in this study [15]

Gene	Primer sequence (5' to 3')	Pathogenicity island	Gene function	Broad action
prgH	F: GCCCGAGCAGCCTGAGAAGTTAGAAA R: TGAAATGAGCGCCCCTTGAGCCAGTC	SPI-1	Type III secretion system apparatus	Invasion of macrophages
sopB	F: CGGACCGCCCAGCAACAAAACAAGAAGAAG R: TAGTGATGCCCGTTATGCGTCAGTGTATT	SPI-1	Type III secreted effector protein	Invasion of macrophages
sopE	F: TCAGTTGGAATTGCTGTGGA R: TCCAAAAACAGGAAACCACAC	SPI-1	Type III secreted effector protein	Invasion of macrophages
invA	F: CTGGCGGTGGGTTTTGTTGTCTTCTCTATT R: AGTTTCTCCCCCTCTTCATGCGTTACCC	SPI-1	Type III secretion system apparatus	Invasion of macrophages
sitC	F: CAGTATATGCTCAACGCGATGTGGGTCTCC R: CGGGGCGAAAATAAAGGCTGTGATGAAC	SPI-1	Iron transport	Invasion of macrophages/iron acquisition
spiC	F: CCTGGATAATGACTATTGAT R: AGTTTATGGTGATTGCGTAT	SPI-2	Type III secretion system	Survival in macrophages
sifA	F: TTTGCCGAACGCGCCCCCACACG R: GTTGCCTTTTCTTGCGCTTTCCACCCATCT	SPI-2	Type III secreted effector protein	Survival in macrophages
misL	F: GTCGGCGAATGCCGCGAATA R: GCGCTGTTAACGCTAATAGT	SPI-3	Involved in intramacrophage survival	Survival in macrophages
orfL	F: GGAGTATCGATAAAGATGTT R: GCGCGTAACGTCAGAATCAA	SPI-4	Adhesin/autotransporter	Survival in macrophages/colonization
pipD	F: CGGCGATTCATGACTTTGAT R: CGTTATCATTCGGATCGTAA	SPI-5	Type III secreted effector-associated with SPI-1 system	Enteritis
iroN	F: ACTGGCACGGCTCGCTGTCGCTCTAT R: CGCTTTACCGCCGTTCTGCCACTGC	NA	Siderophore (iron acquisition)	Associated with iron usage
pefA	F: GCGCCGCTCAGCCGAACCAG R: CAGCAGAAGCCCAGGAAACAGTG	NA	Fimbriae	Movement

(CA-SFM)[13]. Twenty-two antimicrobial disks were used in the following conditions: amoxicillin (25 mg), amoxicillin (20 mg) plus clavulanic acid (10 mg), ticarcillin (75 mg), cephalotin (30 mg), cefoxitin (30 mg), cefotaxime (30 mg), ceftazidime (30 mg), tobramycin (10 mg), amikacin (30 mg), nalidixic acid (30 mg), pefloxacin (5 mg), norfloxacin (10 mg), trimethoprim (1.2 mg) plus sulfamethoxazole (23.75 mg), tetracycline (30 mg), chloramphenicol (30 mg), gentamicin (10 mg), trimethoprim (300 mg), ciprofloxacin (5 mg), spectinomycin (100 mg), streptomycin (10 mg), sulphonamides (200 mg), and nitrofurantoin (300 mg).

PCR virulotyping

PCR virulotyping was conducted on 185 isolates obtained. *Salmonella* cells were lysed and used for screening for the presence of 12 genes thought to be associated with virulence [15] (Table 1). The PCR reactions were performed using ReddyMix PCR Master Mix (ABgene, Epsom, UK). To make a 25 µl reaction, 1.25 µl of primer forward and reverse (10µM each) (Table 1), 9.5 µl of DNA-free water and 0.5 µl template (extracted DNA from *Salmonella* cells) were added to 12.5 µl of ReadyMixPCR containing 0.625 units of Thermo Prime *Taq* DNA Polymerase, 75 mM Tris-HCL, 20 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 0.01% (v/w) Tween 20 and 0.2 mM dNTPs.

Amplification was performed in a thermocycler (TECHNE) as follows: one cycle of 94ºC (3 minutes); 30 cycles of 94ºC (2 minutes), 55ºC (1 minute) and 72ºC (1 minute); and the final cycle at 72ºC for 5minutes. The PCR product (10 µl each) was loaded onto a 2% agarose gel containing 500 ng/µl ethidium bromide at 100V for 1 hour in 1X TBE buffer together with two lines of a 100 bp and a 1 kb ladder (ABgene, Epsom, UK)). The PCR electrophoresis bands were photographed using UV illumination with a gel documentation system (gel

Table 2. Clinical symptoms present in 14 Gambian children with NTS

ID	Age (month)	First diagnosis	Second diagnosis	Serovar
100025	26	diarrhoea	none	Colindale
100132	22	diarrhoea	malaria	sp
102044	26	diarrhoea	malaria	sp
100288	17	diarrhoea	malaria	Colindale
100661	20	diarrhoea	cough, fever more than 38°C	Tado
100385	9	diarrhoea	malaria	Rubislaw
100887	22	diarrhoea	dysentery, cough with difficult breathing	Moualine
102099	26	diarrhoea	belly pain, fever more than 38°C	Colindale
100392	10	healthy	none	sp
100123	20	healthy	none	Chandans
100225	9	healthy	none	Havana
100807	21	healthy	none	Chomedey
100400	9	healthy	none	Chile
100054	10	healthy	none	sp

Doc 2000; Bio-rad). DNA-free water was used as the negative control and *S.* Typhimurium ST19 was used as the positive control.

Statistical analysis

Stata Statistical Software: Release 11 (StataCorp LP, College Station, TX, USA) and MATLAB software (MathWorks, Cambridge, MA, USA) were used for data management and statistical analysis. Binary logistic regression was used to study the association between the presence of certain *Salmonella* virulence genes and resistance to antibiotics with an odds ratios (95% CI) indicating the strength of the associations. We also applied the Fisher's exact test whenever there were not enough numbers of observations to fit logistic regression for certain groups of gene-antibiotics resistance combinations. Thus for such cases p-values reported would be from the Fisher's exact test. We further checked our results for sensitivity to multiple tests and present adjusted p-values using Holm's procedure. Stata 11 (StataCorp. 2009.

Ethical Approval

This research was approved by the Gambia Government /MRC Laboratories joint Ethics Committee under the reference "SCC 1144vs01 26th Nov 2008 Epidemiology and control of *Salmonella* in animals and humans in The Gambia (Upper River Region) and Senegal (Casamance)". Written consent forms were explained to and signed by the participants prior the study.

Results

Among the eight diarrhoea cases, four were co-diagnosed with malaria, two with fever of more than 39°C, and one with dysentery and respiratory infection (Table 2). The prevalence of the 12 different virulence genes studied was high in all serovars (> 70%) except for the *sopE* and *pefA* genes which occurred in 33% and 44% of the isolates respectively. The *invA* gene was most frequently (99.5%) observed. All serovars contained at least two genes of the SPI1 with the majority containing simultaneously all five genes (*prgH, sopB, sopE*, *invA and sitC)* tested (Table 3). *sopE* gene was not detected in the most prevalent serovars isolated from animals in Senegal; namely, *S.* Brancaster, *S*. Agona, *S.* Goelzau and *S.* Kentucky. However, the *sopE* gene was present in all serovars isolated from children with diarrhoea in The Gambia except in one case from which an undetermined serovar was isolated. All *S.* Javiana and *S. Istanbul* which were found in ready-to-eat chicken in restaurants in Senegal contained all the genes except *pefA*.

High levels of resistance were found to trimethoprim-sulfamethoxazole, tetracycline, trimethoprim, streptomycin, and sulfonamides in Senegalese isolates while susceptibility was high to all antibiotics tested (Table 4). We found significant associations ($P < 0.05$) between the presence of virulence genes, most notaby *sopB*, *sitC*, *orfLC*, *pipD*

and *pefA*, and resistance to a number of antibiotics (amoxicillin, ticarcillin, trimethoprim plus sulfamethoxazole, tetracycline, trimethoprim, spectinomycin, streptomycin, sulfonamides and nitrofurantoin). The associated genes belonged to SPI 1, SPI4, SPI5 and plasmid (Table 5).

Discussion

In this study, we found that the *sopE* gene was common in serovars isolated from diarrhoeic children, chicken, sheep and goats in The Gambia. Previous studies have shown that the *sopE* gene was present in strains of *S.* Typhimurium associated with epidemic disease in both humans and animals [16] and therefore the *sopE* gene if expressed may be implicated in diseases in both children and animals in The Gambia. In addition, the *sopB* and *pipD* genes, which are associated with *enteritis* in birds [11,15], were present in 94.1% and 92.4% of all serovars tested, respectively, including those isolated from diarrhoeic children. Furthermore, the *invA* gene was detected in 99.5% of *Salmonella* serovars in this study. The *invA* gene encodes for a protein in the inner and outer membrane, which is essential for the invasion of epithelial cells [17]. The *invA* gene is conserved among *Salmonella* serovars and is a useful marker for molecular detection of *Salmonella* by PCR [18-21]. Serovars Istanbul and Javiana, which were isolated from chicken meat in Senegal, harboured all twelve virulence genes tested except *pefA*. Serovar Istanbul is known to be resistant to commonly used antibiotics in Southern Senegal [13]. High levels of resistance, especially to trimethoprim-sulfamethoxazole, tetracycline, trimethoprim, streptomycin, and sulfonamides, were observed in *Salmonella* serovars isolated in animals from Senegal [13]. Significant associations were found between the presence of some virulence genes, most notably *sopB*, *sitC*, *orfLC*, *pipD* and *pefA*, and resistance to commonly used antibiotics in Senegal and The Gambia. These associations could be explained based on the mechanisms involved in the pathogenicity and the acquisition of resistance genes by *Salmonella*. In fact, in bacteria, the majority of the molecular pathogenicity determinants are located on the chromosome or large virulence-associated plasmids [10,22], whereas antibiotic resistance genes are also often located on extrachromosomal genetic elements or in segments inserted within the chromosome that originate from other genomes [12]. The simultaneous presence in the same bacterial cell of a resident virulence plasmid and resistance gene has been frequently reported in *Salmonella* [12]. It has been reported that resistance plasmids carry genes encoding virulence factors [23]. Also, antibiotic resistance and virulence genes can be linked in the same replicon, or eventually a single determinant can be involved in both virulence and resistance [23]. Although the direct association of virulence and resistance is not determined in this study, much epidemiological data support the idea that, in some cases, antibiotic-resistant organisms may show a decrease in pathogenicity [24-26]. The opposite situation might also occur [27-29].Two of the seven genes, *sopB* and *pipD* associated with resistance in our study, were also associated with enteritis or epidemic disease in both children and animals [11,15,16]. This result shows that our isolates could also exhibit such epidemic potential since these virulence genes were detected in almost all diarrhoeic children.

Conclusion

This study provides a starting point for the development of appropriate molecular tools to study the epidemiology of NTS in Africa. The limitations of our study were mostly related to insufficient numbers of serovars, which could explain the relatively low occurrence of certain genes. Nevertheless, these findings were worrisome, considering that most serovars detected from food and animal sources harboured virulence genes and thus cause diarrhoea in children. Although transmission from animal to man has not yet been elucidated, these results suggest that animals could play an important role in the epidemiology of NTS. The *invA* gene, as shown by several studies, remains a suitable molecular tool to diagnose *Salmonella* in humans as well as in animals and animal products. More detailed studies should focus on various host factors, including age, sex, nutritional status, genetic constitution (including association of virulence and resistance), and the status of the immune system, which affects the outcome of the parasite-host interaction. These further studies would better the understanding of the full mechanism of the pathogenicity of *Salmonella*. Furthermore, these results have shown the important implications of *Salmonella* with regard to the spread and persistence of resistance and virulence genes in *Salmonella* and to the judicious use of antimicrobial agents in humans and animals.

Table 3. Virulence genes detected in *Salmonella* serovars in The Gambia and Senegal

			SPI1					SPI2		SPI3	SPI4	SPI5	plasmid	plasmid	
Origin	**Serovar**	**n**	***prgH***	***sopB***	***sopE***	***invA***	***sitC***	***spiC***	***sifA***	***misL***	***orfL***	***pipD***	***iroN***	***pefA***	Country
chicken meat	Agona	15	15	13	1	15	13	15	15	15	14	15	8	15	Senegal
chicken meat	Brancaster	40	39	40	1	40	4	19	39	38	40	40	38	39	Senegal
chicken meat	Goelzau	28	22	27	5	28	27	28	28	28	27	28	14	14	Senegal
chicken meat	Hadar	19	17	17	5	19	18	18	19	19	13	15	7	0	Senegal
chicken meat	Kentucky	22	22	21	3	22	22	22	22	22	21	16	16	0	Senegal
chicken meat	Poona	8	6	8	2	8	8	8	8	8	8	4	4	2	Senegal
chicken meat	Javiana	1	1	1	1	1	1	1	1	0	1	1	1	0	Senegal
chicken meat	Javiana	1	1	1	1	1	1	1	1	1	1	1	1	0	Senegal
chicken meat	Javiana	1	1	1	1	1	1	1	1	1	1	1	1	0	Senegal
chicken meat	Bandia	1	1	1	1	1	1	1	1	1	1	1	1	0	Senegal
chicken meat	Bessi	1	1	1	1	1	1	1	1	1	1	1	1	0	Senegal
chicken meat	Brunei	1	1	1	1	1	1	1	1	1	1	1	1	0	Senegal
chicken meat	Hull	1	1	1	1	1	1	1	1	1	1	1	1	0	Senegal
chicken meat	Istanbul	1	1	1	1	1	1	1	1	1	1	1	1	1	Senegal
chicken meat	Magherafelt	1	1	1	1	1	1	1	1	1	1	1	1	1	Senegal
chicken meat	Molade	1	1	1	1	1	1	1	1	1	1	1	1	1	Senegal
chicken meat	Oxford	1	1	1	1	1	1	1	1	1	1	1	1	1	Senegal
chicken meat	Rubislaw	1	1	1	1	1	1	1	1	1	1	1	1	1	Senegal
chicken meat	Tamale	1	1	1	1	1	1	1	1	1	1	1	1	1	Senegal
chicken meat	Zanzibar	1	1	1	1	1	1	1	1	1	1	1	1	1	Senegal
chicken meat	sp	3	3	3	3	3	3	3	3	3	3	3	3	3	Senegal
poultry	sp	1	1	1	1	1	1	1	0	1	1	1	1	0	Gambia
poultry	sp	1	1	1	0	1	1	1	1	1	1	1	1	0	Gambia
poultry	sp	1	1	1	1	1	1	1	1	1	1	1	0	0	Gambia
poultry	Tornow	1	1	1	1	1	1	1	1	1	1	1	1	0	Gambia
poultry	Offa	1	1	1	1	1	1	1	1	1	1	1	1	0	Gambia
poultry	Salamae	1	1	1	1	1	1	1	1	1	1	1	1	0	Gambia
poultry	Schwarzengrund	1	1	1	1	1	1	1	1	1	1	1	1	0	Gambia
poultry	sp	1	1	1	1	1	1	1	1	1	1	1	1	0	Gambia
poultry	Poona	1	1	1	1	1	1	1	1	1	1	1	0	0	Gambia

Table 3. Virulence genes detected in *Salmonella* serovars in The Gambia and Senegal (continued)

poultry	Kivu	1	1	1	0	1	1	1	0	1	1	1	1	0	Gambia
poultry	Poona	1	0	0	1	0	1	1	1	0	1	1	1	0	Gambia
poultry	Poona	1	1	0	0	1	1	1	0	0	1	1	0	0	Gambia
poultry	Poona	1	1	1	0	1	1	1	1	1	1	1	0	0	Gambia
poultry	sp	1	1	1	1	1	1	1	1	0	1	1	0	0	Gambia
poultry	sp	1	1	1	1	1	1	1	1	1	1	1	0	0	Gambia
sheep	sp	1	1	1	1	1	1	1	1	1	1	1	0	0	Gambia
sheep	sp	1	1	0	1	1	1	1	1	0	1	1	0	1	Gambia
sheep	sp	1	1	1	1	1	1	1	1	0	1	1	0	0	Gambia
sheep	Johanesburg	1	1	1	0	1	1	1	1	0	1	1	0	0	Gambia
sheep	Lerum	1	1	1	1	1	1	1	0	1	1	1	1	0	Gambia
sheep	Poona	1	1	1	1	1	1	1	0	1	1	1	1	0	Gambia
goat	sp	1	1	0	1	1	1	1	1	0	1	1	1	1	Gambia
healthy child	Chandans	1	1	1	1	1	1	1	1	1	1	1	1	0	Gambia
healthy child	Havana	1	1	1	0	1	1	1	1	1	1	1	1	0	Gambia
healthy child	Chile	1	1	1	1	1	1	1	1	1	1	1	1	0	Gambia
healthy child	Chomedey	1	1	1	1	1	1	1	1	1	1	1	1	0	Gambia
healthy child	sp	1	1	1	0	1	1	1	0	1	1	1	1	0	Gambia
healthy child	sp	1	1	1	1	1	1	1	1	1	1	1	1	0	Gambia
diarrheic child	Sp	1	1	1	1	1	1	1	1	0	1	1	1	0	Gambia
diarrhoeic child	Colindale	1	1	0	1	1	1	1	1	1	1	1	1	1	Gambia
diarrhoeic child	Colindale	1	1	1	1	1	1	1	1	1	1	1	1	1	Gambia
diarrhoeic child	Colindale	1	1	1	1	1	1	1	1	1	1	1	1	1	Gambia
diarrhoeic child	sp	1	1	0	1	1	1	1	1	1	1	1	1	0	Gambia
diarrhoeic child	Tado	1	1	1	1	1	1	1	1	1	1	1	1	0	Gambia
diarrhoeic child	Moulaline	1	1	1	1	1	1	1	1	1	1	1	1	0	Gambia
diarrhoeic child	Rubislaw	1	1	1	0	1	1	1	1	1	1	1	1	0	Gambia
	TOTAL	**185**	**173**	**173**	**62**	**184**	**145**	**163**	**178**	**174**	**176**	**171**	**130**	**85**	
		%	**94.1**	**94.1**	**33**	**99.5**	**78**	**88.1**	**96**	**94.6**	**95.1**	**92.4**	**70**	**44**	

Table 4. Antimicrobial resistance patterns of *Salmonella* isolates used in this study

		Antimicrobials																				
***Salmonella* serovar**	**n**	**AMX**	**AMC**	**TIC**	**CF**	**FOX**	**CTX**	**CAZ**	**TM**	**AN**	**NA**	**PEF**	**NOR**	**STX**	**TE**	**C**	**GM**	**TMP**	**CIP**	**SPT**	**S**	**SSS**	**FI**
Agona	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	3	0
Bandia	2	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	2	1	2	0	1	0
Bessi	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brancaster	40	0	0	0	0	0	0	0	0	0	0	0	0	40	40	0	0	40	0	0	5	40	0
Brunei	1	1	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	1	0
Goelzau	28	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	3	4	2	0
Hadar	19	1	0	1	0	0	0	0	0	0	0	0	0	19	19	0	0	19	0	7	17	19	3
Hull	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Istanbul	4	0	0	0	2	0	0	0	0	0	0	0	0	4	4	0	2	4	0	2	4	4	0
Javiana	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kentucky	22	8	0	8	0	0	0	0	0	0	0	0	0	13	10	0	0	13	0	8	11	0	0
Magherafelt	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Molade	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1	1	0
Oxford	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Poona	8	8	0	7	0	0	0	0	0	0	0	0	0	8	8	0	0	8	0	8	6	7	0
Rubislaw	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tamale	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	1	1	0	0
Zanzibar	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Johannesburg	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Poona	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Chile	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Colindale	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Other Gambian isolates[1]	28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	185	18	0	17	2	0	0	0	0	0	0	0	0	90	85	0	2	89	1	36	54	78	3
%		9.73	0	9.2	1.1	0	0	0	0	0	0	0	0	48.6	46		1.1	48.1	0.5	19	29.2	42.2	1.6

AMX, amoxicillin; AMC, amoxicillin plus clavulanic acid; TIC, ticarcillin; CF, cephalotin; FOX, cefoxitin; CTX, cefotaxime; CAZ, ceftazidime; TM, tobramycin; AN, amikacin; NA, nalidixic acid; PEF, pefloxacin; NOR, norfloxacin; STX, trimethoprim plus sulfamethoxazole; TE, tetracycline; C, chloramphenicol; GM, gentamicin; TMP, trimethoprim; CIP, ciprofloxacin; SPT, spectinomycin; S, streptomycin; SSS, sulfonamides; FI, nitrofurantoin

[1]Other Gambian isolates: Kivu, Lerum, Moualine, Offa, Schwarzengrund, Stanleyville, Tado, Tornow, Give, Sp.

Table 5. Significant P-value of Fisher's exact test for the association virulence gene-antibiotic resistance

Salmonella **Pathogenicity Island**	**Virulence genes**	**Antibiotics resistance**	**Fisher exact P-value**	**Crude Odd Ratio gene (logistic regression)**
SPI 1	*SopB*	STX	0.03	5.18
SPI 1	*SopB*	TE	0.04	4.83
SPI 1	*SopB*	TMP	0.03	5.12
SPI 1	*SopE*	STX	< 0.01	0.40
SPI 1	*SopE*	TE	< 0.01	0.35
SPI 1	*SopE*	TMP	< 0.01	0.37
SPI 1	*SopE*	S	0.001	0.33
SPI 1	*SopE*	SSS	0.0001	0.25
SPI 1	invA	AMX	0.04	0.32
SPI 1	*sitC*	STX	< 0.00001	0.05
SPI 1	*sitC*	TE	< 0.00001	0.04
SPI 1	*sitC*	TMP	< 0.00001	0.05
SPI 1	*sitC*	S	< 0.00001	0.03
SPI 1	*sitC*	SSS	< 0.00001	0.07
SPI 1	*spiC*	STX	< 0.00001	NaN*
SPI 1	*spiC*	TE	< 0.00001	NaN
SPI 1	*spiC*	TMP	< 0.00001	NaN
SPI 1	*spiC*	S	< 0.00001	NaN
SPI 1	*spiC*	SSS	< 0.00001	NaN
SPI 4	*orfL*	FI	< 0.01	0.02
SPI 5	*pipD*	AMX	< 0.001	0.10
SPI 5	*pipD*	TIC	< 0.01	0.14
SPI 5	*pipD*	STX	0.03	0.23
SPI 5	*pipD*	TMP	0.03	0.23
SPI 5	*pipD*	SPT	< 0.001	0.13
SPI 5	*pipD*	S	0.01	0.16
SPI 5	*pipD*	SSS	< 0.01	0.08
plasmid	*pefA*	AMX	0.02	0.23
plasmid	*pefA*	TIC	0.04	0.25
plasmid	*pefA*	STX	0.04	1.88
plasmid	*pefA*	TE	0.04	1.96
plasmid	*pefA*	SSS	0.02	2.20

AMX, amoxicillin; TIC, ticarcillin; STX, trimethoprim plus sulfamethoxazole; TE, tetracycline; TMP, trimethoprim; SPT, spectinomycin; S, streptomycin; SSS, sulfonamides; FI, nitrofurantoin

*NaN: could not be estimated

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