Original Article

Genotypic characterization of antibiotic-resistant *Salmonella Enteritidis* isolates in Dakar, Senegal

Amy Gassama Sow,[1] Abdoul Aziz Wane,[1] Mamadou Hadi Diallo,[1] Cheikh Saad-Bouh Boye,[2] Awa Aïdara-Kane[1]

[1]*Laboratoire de Bactériologie Expérimentale, Institut Pasteur, Dakar, Sénégal;* [2] *Unité de Recherche et des Biotechnologies microbiennes, Faculté de Médecine et Pharmacie, Université Cheikh Anta Diop, Dakar, Sénégal.*

Abstract

Background: It is well established that *Salmonella enterica* is a major cause of food-borne disease worldwide. In Africa, according to the Who Global Salm-Surv country data bank from 2000 to 2002 *Salmonella enterica* serovar Enteritidis was the most common serotype involved in human salmonellosis. In Dakar this serotype of *Salmonella* has been reported as a frequent and an increasing cause of human infection.

Methodology: The genetic determinants of the antimicrobial resistance of 25 selected multiresistant strains of *Salmonella enterica* serovar Enteritidis referred to the National Reference Center for Enterobacteria (NRCE) in Dakar were investigated using molecular techniques.

Results: All strains carried $bla_{TEM\ 1}$ genes. Five harboured three types of class 1 integrons with gene cassettes *dfrA15*, *dfrA1-aadA1* and *dfrA7*. Multiresistance was due to a 23 Kb conjugative plasmid. DNA fingerprinting by macrorestriction of genomic DNA revealed a single related group suggesting that strains might be clonal.

Conclusions: The spread of resistance genes through plasmid transfer plays an important role in the dissemination of antibiotic resistance in enteric pathogens such as *Salmonella Enteritidis*; the risk of transmissibility of antibiotic resistance between different bacterial strains highlights the urgent need to develop strategies to limit the spread of antimicrobial resistance among bacterial enteropathogens.

Key Words: *Salmonella Enteritidis*, Genotypic characterization, Resistance, Senegal.

J Infect Developing Countries 2007; 1(3):284-288.

Received 2 November 2007 - Accepted 21 November 2007.

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Introduction

It is well-established that *Salmonella enterica* is a major cause of food-borne disease worldwide. Over 2,500 serovars have been described to date. In Africa, according to the WHO Global Salm-Surv country data bank from 2000 to 2002, *Salmonella enterica* serovar Enteritidis is the most common serotype involved in human salmonellosis [1]. In Dakar this serotype of *Salmonella* has been reported as a frequent and an increasing cause of human infection by the National Reference Center for Enterobacteria (NRCE: www.pasteur.sn). The development of bacterial resistance to antimicrobial agents is a worlwide concern. In recent years, an increase of antimicrobial resistance has been observed among certain serotypes of *Salmonella enterica* isolates: *S. Enteritidis* appeared simultaneously around the world in the 1980s, and *S.* Typhimurium DT104 appeared in Western Europe and North America in the 1990s [2,3]. The problem is more acute in developing countries where there is uncontrolled use of inexpensive broad-spectrum antibiotics in addition to a crucial lack of means to control the evolution and the spread of resistance. *Salmonella enterica* is causing particular concern because of its increasing prevalence and resistance to multiple antibiotics. Information on *Salmonella* from Africa, together with details of their antibiotic resistance profiles and resistance genes, are limited [4,5,6]. In this study we investigated the genetic determinants of multiresistant *Salmonella Enteritidis* isolated in Dakar.

Materials and Methods

Bacterial isolates

Twenty-five isolates resistant to at least one of the following antibiotics: ampicillin, tetracyclin,

streptomycin, sulfamethoxazol, trimethoprim, and chloramphenicol out of a total of 119 S. *Enteritidis*, received by the Senegalese Reference Center for Enterobacteria during 2001-2002 were included in the study (Table 1). All isolates were identified with the API 20E system (Biomérieux, Marcy l'Etoile, France), and serotyped using the Kauffmann-White scheme [7].

Detection for the presence of bla_{TEM1} *ß-lactamases*

PCR analysis was used to detect the presence of bla_{TEM1}, ß-lactamase with the primers Tem(5'-CAGCGGTAAGATCCTTGAGA-3'and 5'ACTCCCCGTCGTGTAGATAA-3')[8].
Amplification conditions were predenaturation at 94°C, for 5 minutes, followed by 35 cycles of 94°C for 1 minute, 58-60°C for 1 minute, and 72°C for 1 minute, with a final extension 72°C for 10 minutes. Amplified DNA products were resolved by conventional electrophoresis through horizontal 1% agarose gel (GIBCO BRL, Cergy pontoise, France) containing ethidium bromide with 0.5X Tris-Borate-EDTA buffer at 150v; the results were visualised and photographed under a UV light.

Detection and characterization of integrons

Integrons analysis was performed with the following primers: intI1 (5'ACA TGT GAT GGC GAC GCA CGA-3'and 5'-ATT TCT GTC CTG GCT GGC GA-3'), intI 2 (5'-CAC GGA TAT GCG ACA AAA AGG T-3', and 5'GTA GCA AAC GAC TGA CGA AAT G-3'), intI3 (5'-GCC CCG GCA GCG ACT TTC AG-3', and 5'-ACG GCT CTG CCA AAC CTG ACT-3') [9-11], with 50µl of reaction mixture consisting of Taq polymerase buffer, 1.5 mM $MgCl_2$, 200 µM deoxynucleotide triphosphates, 50 pmol of each primer (Proligo, Paris, France), 1U of Taq DNA polymerase, and 25 ng of DNA. PCR amplification was performed in a 2720 thermal cycler (Applied Biosystems, Netherlands) by using the following temperature profiles: predenaturation at 94°C, for 5 minutes, followed by 35 cycles of 94°C for 1 minute, 55-62°C for 1 minute, and 72°C for 1 minute, with a final extension 72°C for 10 minutes. PCR products were analysed by electrophoresis in ethidium bromide-stained agarose gels.

PCR amplification for the characterization of class 1 integron cassettes was performed with primers 5'CS (5'-GGC ATC CAA GCA GCA AG-3') and 3'CS (5'-AAA GCA GAC TTG ACC TGA-3') [12]. The PCR conditions were the same as cited above; PCR products were separated by agarose gel electrophoresis and purified using a QIAquick gel extraction kit (Qiagen, S. A. Courtaboeuf, France).

DNA sequencing

Purified PCR products were sequenced with dye terminator on ABI Prism automatic sequencer as described by the manufacturers; the nucleotide sequences were compared online at the National Center for Biotechnology Information (NCBI) website.

Bacterial conjugations

Mating experiments were carried out on Luria-Bertani broth with *E. coli* C1a resistant to nalidixic acid as recipient strain. Transconjugants were selected on Luria-Bertani agar plates containing ampicillin (100µg/ml), nalidixic acid (50µg/ml), and trimethoprim (5µg/ml). PCR for *intI1*, $bla_{TEM\ 1}$ genes were performed in all transconjugants.

Plasmid analysis

Plasmid DNA was extracted from *Salmonella Enteritidis* isolates and their transconjugants by the method described by Kado and Liu [13], and digested with *BglI* restriction endonuclease (Roche diagnostics GmbH, Mannheim, Germany). The digests were subjected to electrophoresis in a 1% agarose gel at 70V for 3 h. Hind III digests of lambda DNA was used as molecular size markers (Sigma-Aldrich, Germany).

Molecular typing

Isolates were typed using a chromosomal macrorestriction method (PFGE). In brief, bacterial cells were embedded in low-melting-point agarose (Bio-Rad, Marnes-la-Coquette, France) and lysed with lysis buffer containing lysozyme and proteinase K. DNA were digested with 20U of restriction endonuclease *Xba I* (Bio-Rad, Marnes-la-Coquette, France), at 37°C. PFGE was performed with a 1% agarose gel in 0.5X Tris-Borate-EDTA buffer at 10°C by using the Genepath system (Bio-Rad, Marnes-la-Coquette, France). The fingerprinting II software version 3.0 (Bio-Rad, Marnes-la-Coquette, France) was used to interpret chromosomal DNA restriction patterns. Isolates were considered to be genetically related

if their macrorestriction DNA patterns differed by fewer than seven bands [14].

Results

Antimicrobial resistance

All isolates were from human origin (16 were from blood, 6 were from stools, 2 were from urine, and 1 was from pus). Seven antimicrobial resistance profiles were identified. All isolates were resistant to ampicillin (Table 1).

PCR mapping and sequencing antibiotic resistance genes

The detection for the presence of *bla*$_{TEM1}$ genes was positive for all isolates. Sequencing of the PCR products revealed the presence of *bla*$_{TEM\ 1}$ gene.

Class 1 integrons were present in 05 resistant isolates (20%). Neither class 2 nor class 3 integrons were detected. The amplification of the variable region of class 1 integrons revealed that the quasi totality of isolates harboured an inserted DNA of 0.8kb in size; one strain harboured a fragment of 1.5kb. Sequencing and analysis of purified amplicons showed the presence of gene cassettes *dfrA15* or *dfrA7* for the 0.8 kb fragment, and *dfrA1-aadA1* for the 1.5kb fragment; *dfr* and *aadA1* gene cassettes encoding respectively for trimethoprim and streptomycin/spectinomycin resistance (Table 1).

Transfer of antibiotic resistance

Conjugation experiments were successful for all *Salmonella Enteritidis* isolates suggesting that resistance to ampicillin, tetracyclin, streptomycin, sulfamethoxazol, trimethoprim, trimethoprim-sulfamethoxazol and chloramphenicol was transferred to *E. coli* C1a from each of the isolates harboring class 1 integrons and *bla*$_{TEM1}$ genes. Plasmid analysis revealed that the totality of resistant isolates and transconjugants harboured a 23 kb plasmid. The polymerase chain reaction analysis of the plasmid DNA confirmed the transfer of the class 1 integrons and the *bla*$_{TEM1}$ gene suggesting that these resistant determinants were borne by conjugative plasmids.

Molecular typing

One PFGE type was observed suggesting that isolates were closely related.

Table 1. Characteristics of antibiotic-resistant *Salmonella Enteritidis* isolates.

No.	Origin	Year	Antimicrobial resistance phenotypes	Genetic resistance determinants TEM	*intI gene*	*gene cassette*	Plasmid profile (Kb)
01	blood	2001	Amp^R	TEM1	-	-	23
02	blood	2001	Amp^{R}Te^R	TEM1	-	-	23
03	blood	2001	Amp^R	TEM1	-	-	23
04	urines	2001	Amp^R	TEM1	-	-	23
05	urines	2001	Amp^{R}Te^R	TEM1	-	-	23
06	stools	2001	Amp^R	TEM1	-	-	23
07	blood	2001	Amp^R	TEM1	-	-	23
08	pus	2001	Amp^R	TEM1	-	-	23
09	blood	2001	Amp^R	TEM1	-	-	23
10	blood	2002	Amp^{R}Str^R SSS^R SXT^{R}C^R Tmp^R	TEM1	*intI1*	*dfrA1-aadA1*	23-1.4-1.2
11	blood	2002	Amp^{R}SSS^R C^R	TEM1	-	-	23
12	blood	2002	Amp^{R}Str^R SSS^{R}SXT^R Te^{R}Tmp^R	TEM1	*intI1*	*dfrA15*	23
13	stools	2002	Amp^R	TEM1	-	-	23
14	blood	2002	Amp^R	TEM1	-	-	23
15	blood	2002	Amp^R	TEM1	-	-	23
16	blood	2002	Amp^{R}SSS^R SXT^{R}Te^R Tmp^R	TEM1	*intI1*	*dfrA15*	23
17	blood	2002	Amp^R	TEM1	-	-	23
18	Stools	2000	Amp^R	TEM1	-	-	23
19	Stools	2002	Amp^R	TEM1	-	-	23
20	blood	2002	Amp^{R}Str^R SSS^{R}SXT^R Te^{R}Tmp^R	TEM1	*intI1*	*dfrA15*	23-1.5
21	blood	2002	Amp^R	TEM1	-	-	23
22	blood	2002	Amp^R	TEM1	-	-	23
23	blood	2002	Amp^R	TEM1	-	-	23
24	Stools	2002	Amp^R	TEM1	-	-	23
25	Stools	2002	Amp^{R}Str^R SSS^{R}SXT^R Te^{R}C^{R}Tmp^R	TEM1	*intI1*	*dfrA7*	23

Amp, ampicillin ; Te, tetracyclin ; Str, streptomycin ; SSS, sulfamethoxazol, Tmp, trimethoprim ; SXT, trimethoprim-sulfamethoxazol; C, chloramphenicol.

Discussion

In Dakar, in 2001-2002, a high trend in the rate of isolation of *Salmonella Enteritidis* from humans and foods from animal sources with an increase in antibiotic resistance has been reported by the National Reference Center for Enterobacteria (NRCE: www.pasteur.sn). All isolates included in this study were from human origin. The 25 selected isolates were resistant to at least one antibiotic including ampicillin. The main cause of resistance to ampicillin in *Salmonella Enteritidis* is the acquisition of genes encoding TEM-type

betalactamase [4,15]; however, *pse I* has also been shown to cause ampicillin resistance in some strains of *S.* Typhimurium [16]. In Senegal, ampicillin is the cheapest antibiotic, a fact which justifies its use as a first-line drug to treat infectious diseases. The wide use of this antibiotic has led to a high selective pressure for bacterial resistance, and hence the emergence of ß-lactamases in pathogens. This study revealed the presence of class 1 integrons in 5 multiresistant strains (20%) suggesting the major role of these genetic elements in the dissemination of antibiotic resistance. This is in accordance with other reports from the region; integrons have been detected in other serovars of *Salmonella* strains in Dakar [6], and in other Gram negative enteric bacteria isolated in Africa, *Vibrio cholerae*, and *Shigella spp* [17-19]. We found three types of class 1 integron harbouring gene cassettes, *dfrA1-aada1*, *dfrA15* and *dfrA7*, specifying resistance to trimethoprim, streptomycin and / or spectinomycin. The *dfr* gene cassettes were most common, probably due to the intensive use of the trimethoprim in combination with sulfonamides to treat enteric infections. Mating experiments showed that all resistances to antibiotics were transferable and were located on conjugative plasmids. Plasmid analysis revealed that all isolates carried a 23 kb plasmid in which were located different class 1 integrons and TEM1. According to the identical PFGE patterns, these isolates may represent clonal expansion from a common ampicillin resistant ancestor. The integrons harboured by some isolates could be the result of antibiotic selective pressure.

The spread of resistance genes through plasmids transfer plays an important role in the dissemination of resistance genes in Gram-negative enteric pathogens. The risk of transmissibility of antibiotic resistance between different bacterial strains highlights the urgent need to develop strategies to limit the spread of antimicrobial resistance among bacterial enteropathogens.

Acknowledgement

We thank Dr. Coumba Touré-Kane (Laboratoire de Bactériologie-Virologie, hôpital A. Le Dantec, Dakar, Sénégal) for DNA sequencing.

This study was supported by the Pasteur Institute in Dakar.

Conflict of interest

No conflict of interest is declared.

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Corresponding Author: Amy Gassama Sow, Laboratoire de Bactériologie Expérimentale, Institut Pasteur, Dakar, Sénégal, 36, avenue Pasteur BP 220, Dakar, Sénégal. Tel: (221) 839 92 35, Fax: (221) 839 92 36, Email: gassama@pasteur.sn.