Prevalence and characterization of extended-spectrum β-lactamase-producing clinical *Salmonella enterica* isolates in Dakar, Senegal, from 1999 to 2009

D. Harrois[1,2,*], S. Breurec[2,3,*], A. Seck[2], A. Delauné[1], S. Le Hello[1], M. Pardos de la Gándara[1], L. Sontag[1], J.-D. Perrier-Gros-Claude[2], J.-M. Sire[2], B. Garin[2,4] and F.-X. Weill[1]

1) *Institut Pasteur, Unité des Bactéries Pathogènes Entériques, Paris, France,* 2) *Institut Pasteur de Dakar, Unité de Bactériologie Médicale et Environnementale, Dakar, Senegal,* 3) *Institut Pasteur de Bangui, Laboratoire de Biologie Médicale, Bangui, République Centrafricaine and* 4) *Institut Pasteur de Madagascar, Laboratoire de Bactériologie Expérimentale, Antananarivo, Madagascar*

Abstract

A total of 1623 clinical isolates of *Salmonella* belonging to 229 serotypes were received by the Senegalese Reference Center for Enterobacteria from January 1999 to December 2009. The most common serotypes were Enteritidis (19% of the isolates), Typhi (8%), Typhimurium (7%) and Kentucky (4%). A significant increase in the prevalence of resistance to amoxicillin (0.9% in 1999 to 11.1% in 2009) and nalidixic acid (0.9% in 1999 to 26.7% in 2009) was observed in non-typhoidal *Salmonella* serotypes. For critically important antibiotics, notably ciprofloxacin and extended-spectrum cephalosporins (ESCs), the rates of resistance were low: 0.3% and 0.5%, respectively. Seven ESC-resistant *Salmonella* strains and three additional ESC-resistant strains from Senegal (1990) and Mali (2007) were studied to identify the genetic basis of their antibiotic resistance. All ESC-resistant strains produced an extended-spectrum β-lactamase (ESBL). These were CTX-M-15 (n = 6; 2000–2008), SHV-12 (n = 3; 2000–2001) and SHV-2 (n = 1; 1990). A large IncHI2 ST1 pK29-like plasmid was found in six strains (three producing SHV-12 and three CTX-M-15), whereas IncN and IncF plasmids were found in three strains and one strain, respectively. The association of plasmid-mediated quinolone resistance (PMQR) genes *qnrB1* and *aac(6′)-Ib-cr* was found in four ESBL-producing strains, leading to decreased susceptibility and even full resistance to ciprofloxacin (MIC range 0.75–2 mg/L) despite the absence of mutations in the quinolone resistance-determining region (QRDR) of *gyrA*, *gyrB*, *parC* and *parE*. This association of ESBL and multiple PMQR mechanisms within the same strains is therefore a serious concern as it hampers the use of both ESCs and fluoroquinolones for severe *Salmonella* infections.

Keywords: AAC(6′)-Ib-cr, antimicrobial resistance, CTX-M, extended-spectrum β-lactamase, IncHI2 plasmid, IncN plasmid, Qnr, *Salmonella enterica*, Senegal, SHV

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Corresponding author: F.-X. Weill, Unité des Bactéries Pathogènes Entériques, Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris Cedex 15, France

E-mail: francois-xavier.weill@pasteur.fr

*These authors contributed equally.

Introduction

Human *Salmonella* infections are generally either typhoid fever, a systemic disease caused by *S. enterica* serotypes Typhi, Paratyphi A, Paratyphi B (non-*d*-tartrate-fermenting variant) and Paratyphi C, or gastroenteritis caused by a large number of non-typhoidal *Salmonella* (NTS) serotypes. Typhoidal serotypes are human-restricted whereas NTS have large animal reservoirs. Although most salmonellosis due to NTS is self-limiting, serious complications, including systemic infection and death, can occur. Such infections have consistently been reported as a leading cause of bacteraemia in Africa and are associated with a high risk of death [1]. As rates of resistance to all classes of antibiotics have increased throughout the world, conventional antibiotics such as ampicillin, chloramphenicol and cotrimoxazole are no longer

the appropriate choices and extended-spectrum cephalosporins (ESCs) and fluoroquinolones have become standard for first-line empirical treatment in children and adults, respectively [2]. Recently, ESC-resistant (ESC^R) *Salmonella* populations have emerged and spread over all continents, including Africa [2–15]. This resistance is mainly mediated by acquired extended-spectrum β-lactamase (ESBL) genes carried by mobile genetic elements such as plasmids and transposons. This situation is of great concern, as ESBL enzymes can hydrolyze almost all β-lactams (except carbapenems and cephamycins), and are frequently associated with genes conferring resistance to several other classes of antibiotics. Recently, plasmid-mediated quinolone resistance (PMQR) has emerged in Enterobacteriaceae. Four PMQR mechanisms have been described: Qnr, AAC(6′)-Ib-cr (AACA4-cr), OqxAB and QepA, which mediate target protection (Qnr), drug modification (AAC(6′)-Ib-cr) and drug efflux (OqxAB and QepA) [16]. These mechanisms result in an increase of the minimum inhibitory concentration (MIC) of fluoroquinolones, thereby facilitating the selection of mutants with higher levels of resistance in the presence of quinolones through sequential chromosomal mutations in genes coding for the target enzymes, DNA gyrase and/or DNA topoisomerase IV [16].

Few data are available regarding the prevalence of ESC^R *Salmonella* strains in Africa or their antibiotic resistance gene content and their genetic support. Such information is important for an understanding of the spread of multidrug-resistant *Salmonella* spp.. Here, we report the prevalence of resistance to antibiotics in *Salmonella* spp. isolated from Senegalese patients between 1999 and 2009, and the genetic basis for this antibiotic resistance. Three additional ESC^R strains from Senegal (1990) and from Mali (2007) were also included to provide a better description of circulating ESBL-producing *Salmonella* strains in West Africa.

Materials and Methods

Bacterial strains, serotyping and susceptibility

A total of 1623 *S. enterica* clinical isolates were received between January 1999 and December 2009 by the Senegalese Reference Center for Enterobacteria (Institut Pasteur, Dakar, Senegal) from four major public and private clinical laboratories (Hôpital Aristide Le Dantec, Hôpital Principal, Institut Pasteur and Bio24) located in Dakar. If more than one isolate with the same serotype and antimicrobial resistance phenotype was recovered from the same patient, only the first was included. Epidemiological data (date and site of isolation, age and gender of the patient) were recorded for each ESC^R isolate. ESC^R *Salmonella* strains from other collections were also included in this study: one strain (09-7364) isolated in Senegal in 1990 (Poitiers University Hospital collection, France) and two strains (07-0319, 07-1331) acquired in Mali in 2007 (the collection of the French National Reference Center for *Salmonella*, Institut Pasteur, Paris).

Strains were serotyped on the basis of somatic O and both phase 1 and phase 2 flagellar antigens by agglutination tests with antisera (Bio-Rad, Marnes-La-Coquette, France) as specified by the White-Kauffmann-Le Minor scheme [17]. Antibiotic susceptibility to amoxicillin, amoxicillin-clavulanic acid, ticarcillin, cefalotin, cefoxitin, cefotaxime, ceftazidime, amikacin, tobramycin, gentamicin, nalidixic acid, ciprofloxacin, chloramphenicol, sulphonamides, cotrimoxazole and tetracycline was determined by the disk diffusion method on Mueller-Hinton agar (Bio-Rad) according to the guidelines of the French Society for Microbiology. (http://www.sfm-microbiologie.org/UserFiles/file/CASFM/casfm_2011.pdf).

All strains of *Salmonella* showing resistance to cefotaxime and/or ceftazidime were selected for further analysis. Additional testing was carried out on these strains: (i) susceptibility to piperacillin, piperacillin/tazobactam, imipenem and streptomycin was determined by the disk diffusion method as described above; (ii) they were tested for an ESBL enzyme by the double disk synergy method [18]; and (iii) MICs for nalidixic acid, ciprofloxacin, ceftriaxone and ceftazidime were determined by using Etest strips (bioMérieux, Marcy L'Etoile, France). The CA-SFM cut-off values were used for categorization. Susceptible strains were defined by MIC $\leq$ 8 mg/L for nalidixic acid, MIC $\leq$ 0.5 mg/L for ciprofloxacin, MIC $\leq$ 1 mg/L for ceftriaxone and MIC $\leq$ 4 mg/L for ceftazidime, and resistant strains by MIC $>$ 16 mg/L for nalidixic acid, MIC $>$ 1 mg/L for ciprofloxacin, MIC $>$ 2 mg/L for ceftriaxone and MIC $>$ 4 mg/L for ceftazidime.

Characterization of resistance determinants in ESC^R strains

Total DNA was extracted using the Instagene™ Matrix kit (Bio-Rad) according to the manufacturer's recommendations. The resistance genes, bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M}$, $bla_{OXA\text{-}1}$ group, *qnrA*, *qnrB*, *qnrS*, *qnrD*, *aac(6′)-Ib* and *qepA*, were amplified by PCR from DNA from all ESC^R strains as described previously [5,19]. For all *qnr*-positive ESC^R strains, the quinolone resistance-determining region (QRDR) of *gyrA*, *gyrB*, *parC* and *parE* (encoding subunits of the DNA gyrase and topoisomerase IV) was sequenced, as described previously [19]. The nucleotide and deduced amino acid sequences were analysed and compared with sequences available through the Internet on the National Center for Bio-technology Information web site (http://www.ncbi.nlm.nih.gov).