Prevalence and Characterization of Extended-spectrum Beta-lactamase-producing Clinical *Salmonella enterica* Isolates in Senegal from 1999 to 2009

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ABSTRACT (262)

A total of 1,623 clinical isolates of *Salmonella* belonging to 229 serotypes, mainly from stools (78.6%) and blood (11.7%), were received by the Senegalese Reference Center for Enterobacteria from January 1999 to December 2009. The most common serotypes were Enteritidis (18.5% of the isolates), Typhi (7.8%), Typhimurium (6.4%), and Kentucky (4.1%). During the study period, an increase in prevalence of resistance to certain antibiotics, and in particular amoxicillin (0.9% in 1999 to 11.1% in 2009), cotrimoxazole (5.4% in 1999 to 8.9% in 2009) and nalidixic acid (0.7% in 1999 to 26.7% in 2009), was observed in non-typhoidal *Salmonella* (NTS) serotypes. For critically important antibiotics, notably ciprofloxacin and extended-spectrum cephalosporins (ESCs), the rates of resistance were low: 0.4% and 0.5%, respectively. Seven ESCs-resistant *Salmonella* strains, and three additional ESCs-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 beta-lactamase (ESBL). The ESBL $bla_{\text{CTX-M-15}}$ gene, first detected in 2000, was present in all the five strains isolated between 2007 and 2009, whereas the ESBL $bla_{\text{SHV-2}}$ and $bla_{\text{SHV-12}}$ genes were detected in the older strains. Three strains of different serotypes carried $bla_{\text{CTX-M-15}}$, *qnrB1* and *aac(6')-Ib-cr* genes on a large (280-340 kb) IncHI2 plasmid, suggesting that this plasmid is circulating in West Africa. The accumulation of plasmid-mediated quinolone resistance (PMQR) determinants, including both *qnrB1* and *aac(6')-Ib-cr*, in some ESBL-producing strains, leads 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*.

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, and 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 are self-limiting, chronic fecal carriage and serious complications, including systemic infection and death, can occur. Timely treatment with appropriate antibiotics is essential for reducing the mortality associated with typhoid fever and also for the treatment of invasive NTS infections. Such infections have consistently been reported as a leading cause of bacteremia in Africa and are associated with a high risk of death (1, 2). 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 (3). Recently, ESC-resistant (ESC^R) *Salmonella* populations have emerged and spread on all continents, including Africa (4-21). This resistance is mainly mediated by acquired extended-spectrum beta-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 beta-lactams (except carbapenems and cephamycins), and are frequently associated with genes conferring resistance to several other classes of antibiotics. CTX-M has emerged as the dominant ESBL family in *Salmonella* spp. strains, although TEM and SHV enzymes are also common (22). Recently, plasmid-mediated quinolone resistance (PMQR) has emerged in Enterobacteriaceae. Three PMQR mechanisms have been described: Qnr, AAC(6')-Ib-cr (AACA4-cr) and QepA, which mediate target protection, drug modification, and drug efflux, respectively. These mechanisms result in an increase of the

minimum inhibitory concentration (MIC) of quinolones, thereby facilitating the selection of mutants with higher levels of resistance in the presence of quinolones through chromosomal mutations in genes coding for the target enzymes, DNA gyrase and/or DNA topoisomerase IV (23).

Few data are available for the prevalence of ESC^R *Salmonella* strains in Africa, or for their antibiotic resistance gene content and their genetic environment. 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 1,623 *S. enterica* clinical isolates were received by the Senegalese Reference Center for Enterobacteria (Institut Pasteur, Dakar, Senegal) from private and public clinical laboratories between January 1999 and December 2009. 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), and the time between admission and sample collection for culture in cases of hospitalization were recorded for each ESC^R isolate. Isolates were considered to be community-acquired if recovered by culture from a sample obtained within 48 h of admission.

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 (24). Antibiotic susceptibility to amoxicillin, amoxicillin-clavulanic acid, ticarcillin, cefalotin, cefoxitin, cefotaxime, ceftazidime, amikacin, tobramycin, gentamicin, nalidixic acid, ciprofloxacin, chloramphenicol, sulfonamides, 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). Isolates were categorized as susceptible, intermediate or resistant according to CA-SFM cut-off values. The cut-off values used for nalidixic acid, ciprofloxacin, ceftriaxone, and ceftazidime were slightly different from those determined by the Clinical and Laboratory Standards Institute (CLSI) (M100-S22): susceptible strains were defined by MIC $\leq$ 8 mg/L for nalidixic acid (CLSI, $\leq$ 16 mg/L), MIC $\leq$ 0.5 mg/L for ciprofloxacin (CLSI, $\leq$ 0.06 mg/L), MIC $\leq$ 1 mg/L for ceftriaxone (similar to the CLSI value) and MIC $\leq$ 4 mg/L for ceftazidime (CLSI, $\leq$ 1

mg/L), and resistant strains by MIC >16 mg/L for nalidixic acid (similar to the CLSI value), MIC > 1 mg/L for ciprofloxacin (CLSI, ≥ 1 mg/L), MIC >2 mg/L for ceftriaxone (similar for CLSI) and MIC > 4 mg/L for ceftazidime (CLSI, ≥ 16 mg/L). ESC^R *Salmonella* strains from other collections were also included in this study: one strain belonging to serotype Miami isolated in Senegal in 1990 (Poitiers University Hospital collection, France) and two strains belonging to serotypes Havana and Telelkebir acquired in Mali in 2007 (the collection of the French National Reference Center for *Salmonella*, Institut Pasteur, Paris).

MICs for nalidixic acid, ciprofloxacin, ceftriaxone, and ceftazidime for all ESC^R strains were determined using Etest strips (bioMerieux, Marcy L'Etoile, France). In addition, susceptibility to piperacillin, piperacillin/tazobactam, imipenem, streptomycin and spectinomycin was determined by the disk diffusion method as described above. Production of an ESBL enzyme was detected by the double disk synergy method (25).

Characterization of resistance determinants in ESC^R strains

Total DNA was extracted using the Instagene^{TM} Matrix kit (Bio-Rad) according to the manufacturer's recommendations. The resistance genes, *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX-M}$, *bla*$_{OXA-1}$ group, *qnrA*, *qnrB*, *qnrS*, *qnrD*, *aac(6')-Ib*, *qepA*, *cat*, *tet*A, *aadA1*, and class 1 integron gene cassettes, were amplified by PCR from DNA from all ESC^R strains as described previously (7, 8, 26-31). The quinolone resistance-determining region (QRDR) of *gyrA*, *gyrB*, *parC* and *parE* (encoding subunits of the DNA gyrase and the topoisomerase IV), was amplified by PCR from DNA from all *qnr*-positive ESC^R strains as described previously (32). The PCR products were sequenced at the "Plateforme de Génotypage des Pathogènes et Santé Publique, PF8" (Institut Pasteur, Paris, France). The nucleotide and deduced amino acid sequences were analyzed and compared to sequences available through the Internet at the National Center for Bio-technology Information web site (http://www.ncbi.nlm.nih.gov).

137

138 **Molecular typing**

139 Pulsed-field gel electrophoresis (PFGE) of *Xba*I-digested chromosomal DNA was
140 performed for ESC^R strains of serotypes Kentucky and Agona, as previously described (8)

141

142 **Resistance transfer determination of ESC^R strains**

143 Resistance transfer experiments were carried out on solid media using either
144 *Escherichia coli* K12 J5 resistant to sodium azide or *E. coli* C1a resistant to nalidixic acid as
145 the recipient strain (7, 14). Transconjugants were selected on Drigalski agar (Bio-Rad)
146 supplemented with sodium azide (500 mg/L) and ceftriaxone (2 mg/L) or ceftazidime (4 mg/L)
147 or ciprofloxacin (0.06 mg/L). Three *E. coli* transconjugants were arbitrarily selected in each
148 experiment.

149

150 **Plasmid analysis**

151 Plasmid DNA extracted by alkaline lysis (33) was analyzed by electrophoresis in 0.8%
152 agarose gels. We used S1 nuclease treatment and PFGE to determine the sizes of bacterial
153 plasmids, in parental and transconjugant strains, as described previously (34). PCR based
154 replicon-typing analysis was performed as described previously (35). The 18 primer pairs
155 targeting FIA, FIB, FIC, HI1, HI2, I1, L/M, N, P, W, T, A/C, K, B/O, X, Y, F, and FII
156 replicons were used in separate PCRs. For IncHI2 plasmids, a plasmid backbone analysis was
157 performed by PCR mapping as described previously (36).

158

159 **Exploration of the region upstream from *bla*$_{CTX\text{-}M\text{-}15}$, *bla*$_{SHV\text{-}12}$, and *qnrB1* genes**

To search for an IS*Ecp1* element upstream from $bla_{CTX\text{-}M\text{-}15}$, we used primers IS*Ecp*1U1 (5'-AAAAATGATTGAAAGGTGGT-3'; corresponding to positions 1,545 to 1,564 of IS*Ecp1*) (37) and CTX-M-R (5'-CGC(A/G)ATATC(A/G)TTGGTGGTG-3') (7). To search for an IS*26* element upstream from IS*Ecp1* and $bla_{CTX\text{-}M\text{-}15}$, we used primers IS26-F (5'-AAGGCCGGCATTTTCAGCGT-3'; located at bp 77 to 96 of IS*26*) (37) and CTX-M-R. To search for an IS*26* element usptream from $bla_{SHV\text{-}12}$, IS26-U (5'-AGCGGTAAATCGTGGAGTGA-3'; corresponding to positions 211 to 192 of IS*26*) (37) and SHV-INT-R (5'- GATTTGCTGATTTCGCTCGG-3') (26) were used. PCR amplification was carried out in 50µl reaction mixtures containing of 5µl of 10X PCR buffer, 1.25U of AmpliTaq Gold® DNA Polymerase (Applied Biosystem, Forster city, USA), 200 µM deoxynucleosidetriphosphates, 0.25µM each primer, and 50ng of microbial genomic DNA (Matrix DNA, 2µL). The PCR conditions included an initial denaturation step at 94°C for 10 min, followed by 35 cycles at 94°C for 1 min, an annealing step at 50°C for IS*Ecp1* and 55°C for IS*26* for 1 min, an amplification step at 72°C for 1min 30, and an extension step at 72°C for 10 min. PCR products were analyzed on 1.5% agarose gel stained with ethidium bromide and by DNA sequencing.

The presence of gene *orf1005* (GenBank accession number AF174129) upstream from *qnrB1* (GenBank accession number DQ351241) was investigated in *qnr*-positive strains using primers, Orf1005_ENV_F (5'-AGGTGCAGTACTTCAGCTGC-3') and qnrB1_ENV_R (5'-GCCAGAGCCATATTTGTACC-3'). The Expand Long range dNTPack kit (Roche) was used for PCR according to the manufacturer's recommendations.

184 **RESULTS**

185

186 **Serotype distribution and antimicrobial susceptibility data**

187 A total of 1,623 independent isolates of *Salmonella* belonging to 229 serotypes were
188 collected during the 10-year study period. The most common serotypes were Enteritidis (18.5%
189 of the isolates), Typhi (7.8%), Typhimurium (6.4%) and Kentucky (4.1%) (Table 1). Most of
190 the isolates were from stools (n=1281, 78.6%) and blood (n=191, 11.7%). Among *Salmonella*
191 blood isolates, 118 (61.8%) were NTS with Enteritidis (30.5% of the isolates) and
192 Typhimurium (15.8%) being the predominant serotypes.

193 Overall, NTS isolates had low or moderate resistance to antibiotics. However, an
194 increase in the prevalence of resistance to certain antibiotics, and in particular amoxicillin
195 (0.9% in 1999 to 11.1% in 2009), cotrimoxazole (5.4% in 1999 to 8.9% in 2009, peaking in
196 2008 with 17.4%) and nalidixic acid (0.9% in 1999 to 26.7% in 2009), was observed during the
197 study period (Table 2). Six (0.4% of the total) NTS isolates belonging to serotypes Keurmassar
198 (2000, n=1) (5, 9), Enteritidis (2007, n=2) and Kentucky (2009, n=3) were resistant to
199 ciprofloxacin (MIC in progress); one of these isolates was ESC^R (Keurmassar). All the serotype
200 Typhi (n=127), Paratyphi A (n=19) and Paratyphi B (n=1) isolates were susceptible to all
201 antibiotics tested, except for five serotype Paratyphi A isolates: three were resistant to
202 tetracycline, one was resistant to amoxicillin, and another one to cotrimoxazole. Eight NTS
203 strains (0.5% of the total) belonging to serotypes Keurmassar (n=1, 2000) (5, 9), Kentucky
204 (n=2, 2000 and 2002) (7), Agona (n=2, 2000 and 2001), Grumpensis (n=1, 2008),
205 Typhimurium (n=1, 2008), and Carmel (n=1, 2009) were resistant to all the β-lactams tested,
206 except for cefoxitin, piperacillin-tazobactam and imipenem (Table 3). Both serotype Kentucky
207 strains displayed identical PFGE profiles and antimicrobial susceptibility profiles (7), whereas
208 the two serotype Agona strains had two similar but not identical PFGE profiles (not shown) and

209 different antimicrobial susceptibility profiles. Therefore, only one serotype Kentucky strain but
210 both serotype Agona strains were selected for further molecular investigation. These seven
211 ESC^R strains, as well as three additional ESC^R strains from Mali and Senegal (Miami, Havana
212 and Telelkebir), scored positive in double-disk synergy tests and showed high resistance to all
213 classes of antibiotics: five were resistant to nalidixic acid and three of these five were resistant
214 to ciprofloxacin (MIC 1.5-2 mg/L); and three were resistant to all but one of the
215 aminoglycosides (amikacin) tested.
216
217 **Resistance determinants in ESC^R strains**
218 Among the 10 ESC^R isolates, the *bla*$_{\text{CTX-M-15}}$ gene was detected in all of those isolated
219 between 2007 and 2009 (n=5), and also in one strain isolated in 2000, whereas *bla*$_{\text{SHV-12}}$ was
220 detected in three strains isolated in 2000 and 2001, and *bla*$_{\text{SHV-2}}$ in the oldest strain from 1990
221 (Table 3).
222 The *qnr* gene was present in five ESC^R isolates (62%): two were *qnrB2* and three were
223 *qnrB1*. The *qnrB1* gene was associated with *bla*$_{\text{CTX-M-15}}$ strains, and *qnrB2* with *bla*$_{\text{SHV-12}}$
224 strains. No *qnr* was found in the oldest strain, that produces SHV-2. The five *qnr*-positive
225 strains had wild-type alleles in the QRDR of *gyrA*, *gyrB*, *parC* and *parE*. They displayed MICs
226 to nalidixic acid in the range 24 to 64 mg/L, and MICs to ciprofloxacin of 0.25 to 2 mg/L.
227 According to CLSI breakpoints, three were classified as resistant to ciprofloxacin (MICs 1.5-2
228 mg/L) and two as intermediate (MIC of 0.25 or 0.75 mg/L) (Table 3). The presence of both *qnr*
229 and *aac(6')-Ib-cr* was associated with higher MICs to ciprofloxacin (MICs 0.75-2 mg/L).
230
231 **Genetic support and environment of resistance determinants**
232 All the ESC^R isolates were found to harbor transferable β-lactam resistance determinant
233 genes on conjugative plasmids (Table 3). The *bla*$_{\text{CTX-M-15}}$ genes were located on plasmids of the

234 incompatibility groups HI2 (n=3), N (n=2) and Frepb (n=1). The IS*Ecp1* insertion sequence
235 was found upstream from the *bla*$_{\text{CTX-M-15}}$ gene. Only the serotype Carmel strain yielded a PCR
236 product (≈2.1 kb) with the primers IS26-F and CTX-M-R indicating that an IS*26* was located
237 upstream from a partial IS*Ecp1* (≈ 500 bp) in this strain.
238 Both *qnrB1* and the *aac(6')-Ib-cr* genes were on large IncHI2 plasmids (280-340 kb)
239 also carrying the *bla*$_{\text{CTX-M-15}}$ gene. The *qnrB1* was found downstream from Orf1005 that
240 encodes a putative transposase, as observed in the first *K. pneumoniae* isolates from southern
241 India in the early 2000s (38). Two of these *qnrB1*-positive isolates produced CTX-M-15, and
242 one SHV-12. The genes *bla*$_{\text{OXA-1}}$, *bla*$_{\text{TEM}}$, *tet*A (encoding tetracycline resistance), *cat* (encoding
243 chloramphenicol acetyltransferase) and a*adA1* (encoding resistance to streptomycin and
244 spectinomycin) were also present on the large IncHI2 plasmid. The *aadA1* gene was integrated
245 as a gene cassette into a class 1 integron. Analysis of the plasmid backbone showed that the
246 three IncHI2 plasmids had a same profile (PCR-positive for the following targets: *smr10-11*,
247 *smr207-208*, *smr239-240*, O1R_160, *terF*, and *arsB*).
248 The *S. enterica* serotype Agona and Keurmassar strains isolated in 2000 and 2001,
249 carried *bla*$_{\text{SHV-12}}$ and *qnrB2* on two different plasmids belonging to the IncHI2 group. The
250 serotype Keurmassar strain also carried the *aac(6')-Ib-cr* gene on the same IncHI2 plasmid.
251 The *S. enterica* serotype Miami strain isolated in 1990 had a *bla*$_{\text{SHV-2}}$ gene on an IncN plasmid.
252 The IS*26* insertion element was found upstream from every SHV-encoding gene.
253 The *qnr* genes were transferred by conjugation into *E. coli* recipients. The MICs of
254 ciprofloxacin for the five *E. coli* transconjugants were 1- to 6-fold lower than those for the
255 corresponding parental strains (Table 3). Both *aac(6')-Ib-cr* and *qnr* genes were transferred
256 from three of the four donors. The resulting transconjugants displayed resistance to classes of
257 antibiotics other than ESCs and quinolones, indicating that the corresponding resistance genes
258 were also cotransferred (Table 3).

DISCUSSION

In total, 1,623 non-duplicate *Salmonella* spp. isolates were analyzed during the study period. Overall, the resistance rates to all antibiotic classes were low or moderate although there was a worrying increase in the prevalence of resistance to first-line antibiotics during the study period. The prevalence of ESC^R isolates in our study was 0.5%, consistent with previous studies elsewhere in the world (0 to 2.4%) (22, 39-41). The epidemiology of antibiotic-resistant *Salmonella* spp. isolated from humans has been poorly documented in Africa. National surveillance systems are primarily based on a network of clinical laboratories that refer *Salmonella* isolates to public health laboratories for identification and susceptibility testing. Such systems are essential for the detection and monitoring of antibiotic resistance. National laboratory-based surveillance systems are lacking in most countries with inadequate healthcare systems. Consequently, many studies in Africa are not appropriately representative of the epidemiological situation nationally. Despite these limitations, the number of new cases of ESC^R *Salmonella* strains (novel associations of serotype, enzyme and country) seem to have increased across Africa since the first description in 1988 in Tunisia (4). So far, 23 different *Salmonella* serotypes displaying resistance to ESCs have been described in studies of human isolates. Typhimurium is the most frequently isolated serotype in ten countries in West Africa (Senegal and Mali) (5, 7, 8, 9, 16, 17), Maghreb (Algeria, Tunisia and Morocco) (4, 6, 11, 15, 18), East Africa (Ethiopia and Tanzania) (12, 14, 19, 20), South Africa (South Africa and Zambia) (10, 13, 21) and Central Africa (Central African Republic) (S. Breurec, unpublished data). ESBL-producing *Salmonella* can be acquired in the community (7, 10, 15, 19) or in hospitals (6, 11, 12, 18, 42), in particular in pediatric wards and neonatology units. Several reports also mention internationally adopted children from Mali or Ethiopia as carriers of ESBL-producing *Salmonella* of various serotypes (8, 14, 16, 17, 20). The such strains might

may have been selected in the orphanages where the children had stayed before adoption: the prevailing conditions, like overcrowding and high ESCs pressure, may have favored such strains. However, a study performed outside orphanages found that one of the ESBL-producing *Salmonella* strains (serotype Concord) circulating in the orphanage was also circulating in the general population of Ethiopia (19). In our study, the $bla_{\text{SHV-12}}$ gene was detected in three strains isolated in early 2000s, $bla_{\text{SHV-2}}$ in the oldest strain from 1990, and $bla_{\text{CTX-M-15}}$ gene in all the strains isolated since mid 2000s. This shift from TEM/SHV to CTX-M, and in particular to CTX-M-15, is in accordance with other epidemiological studies of ESBL-producing *Salmonella* spp. in West Africa (14, 16, 17, 20). CTX-M-15 ESBL, first detected in 1999 in India, is currently recognized as the most widely distributed ESBL in *Enterobacteriaceae* including *Salmonella*, even on the African continent, where it has replaced TEM and SHV ESBLs. Another matter of concern is the accumulation of PMQR determinants, such as both *qnrB1* and *aac(6')-Ib-cr*, in some ESBL-producing strains; these determinants lead to decreased susceptibility and even full resistance to ciprofloxacin (MIC range 0.75-2 mg/L) despite the absence of mutations in the QRDR.

The ESBL $bla_{\text{CTX-M-15}}$ gene was carried on plasmids of three different incompatibility groups, suggesting that the dissemination of $bla_{\text{CTX-M-15}}$ among *Salmonella* spp. in Senegal and Mali is not due to a single type of plasmid. The IS*Ecp1* insertion sequence was found upstream from the $bla_{\text{CTX-M-15}}$ gene as previously described (7, 43). This element belongs to the IS*1380* family, and can mobilize the adjacent $bla_{\text{CTX-M-15}}$ gene (43), suggesting a role in its dissemination. Furthermore, IS*Ecp1* harbors promoter sequences known to be involved in $bla_{\text{CTX-M-15}}$ gene expression (43). The $bla_{\text{CTX-M-15}}$, *qnrB1* and *aac(6')-Ib-cr* genes were carried by a similar IncHI2 plasmid present in three different serotypes (Grumpensis, Telelkebir and Havana), demonstrating that this plasmid is circulating in West Africa. However, this plasmid is different from the CTX-M-15/IncFII plasmid present in the ST131 *E. coli* clone disseminated

309 worldwide (44). Frequent association of *qnrB,* $bla_{\text{CTX-M-15}}$ and *aac(6')-Ib-cr* genes has been
310 described in *K. pneumoniae* isolates from Maghreb (Morocco), West Africa (Senegal, Ivory
311 Coast), Central Africa (Cameroon), East Africa (Madagascar) (45), consistent these resistance
312 determinant genes being carried together on the same plasmid as described in *K. pneumoniae*
313 strains from Nigeria (46). However, these two studies did not determine the incompatibility
314 groups of the plasmids involved. Further studies on the genetic environment of antibiotic
315 resistance gene from ESC^R *Enterobacteriaceae* strains in Africa are needed as part of the global
316 epidemiological surveillance of plasmid-mediated ESC resistance.
317 The presence of ESC^R *Salmonella* strains in humans raises the question of their origin.
318 The emergence of antimicrobial resistance in NTS isolates from humans is thought to be mostly
319 due to the use of antimicrobial agents in animal feed (47, 48). Although it is difficult to resolve
320 the issue, this is probably also the case in Senegal as ESC^R *Salmonella* strains have emerged in
321 both food and in humans (5, 9, 15). Certain conditions prevailing in Africa may favor the
322 emergence and spread of these ESC^R *Salmonella* strains in humans. First, antibiotics are used
323 extensively for animal growth and therapy in Senegal (9) and lack of good hygiene practices in
324 most of farms and abattoirs may favor transmission of these resistant strains from animals and
325 food to humans (49). Second, poor hygiene and sanitary conditions, overcrowding, and
326 antibiotic selective pressure increase the risk of interindividual transmission as illustrated by
327 the spread of ESBL-producing *Salmonella* spp. in African hospitals and orphanages. Four of
328 our ESC^R isolates were isolated from patients considered to have hospital-acquired infections,
329 although this must be interpreted with caution as patients in African hospitals are not
330 systematically sampled for bacteriological culture within the first 48 h of admission as a
331 consequence of organizational problems. Furthermore, the permanent presence of friends and
332 relatives in African hospitals, and the transfer of some healthcare responsibilities to them,
333 increases the risk of ESC^R *Salmonella* circulation between the community and hospitals. Third,

most African countries have no surveillance systems, and specific measures to control outbreaks are either not undertaken or implemented too late.

The data reported here add to the knowledge of the circulation of multidrug-resistant *Salmonella* populations in West Africa. Although rare, resistance to ESC and ciprofloxacin is present. Therefore, it seems important to continue monitoring antimicrobial susceptibility in *Salmonella* isolates from humans, foodstuffs and food animals in Senegal.

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TRANSPARENCY DECLARATIONS

All the authors declare that they have no conflicts of interest.

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