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Original Article

High prevalence of trimethoprim-resistance cassettes in class 1 and 2 integrons in Senegalese *Shigella* spp isolates

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Abstract

Background: Integrons have a well-established role in the dissemination of resistance among Gram-negative pathogens and are thus a useful marker of antibiotic resistance. Shigellae are noteworthy for their multiple drug resistance, having gradually acquired resistance to most widely use and inexpensive antimicrobial drugs.

Methodology: A total of 32 *Shigella* strains belonging to serotypes *flexneri*, *dysenteriae*, and *boydii* 20, a new *Shigella* serovar, resistant to at least four antibiotics were analyzed by molecular techniques.

Results: Class 1 integrons were the most prevalent (92.8%); class 2 integrons were found in 16 strains (57.1%). Fifty percent of the strains harboured both class 1 and 2 integrons (*intI1* and *intI2* genes); this combination of integrase genes was most prevalent in *S. boydii* 20 and *S. dysenteriae* strains. The class 1 integrons detected contained *dfr* and *aadA* cassettes, alone or in combination (*dfrA5*/*dfrA15*, or *dfrA15-aadA1*, *dfrA1-aadA2*), and an atypical cassette array with an insertion sequence (*oxa30-aadA1*-IS*1*). For class 2 integrons, we detected either the same cassettes as those found in Tn*7* (*dfrA1-sat1-aadA1-orfX*) or truncated class 2 integrons without *aadA1* or *orfX*. The *tns* genes were absent from all class 2 integrons.

The distribution of integrons among RAPD profiles and serotypes revealed a clonal spread of integrons into serotypes and a transfer of integrons between different serotypes.

Conclusions: The detection of integrons in a new *Shigella* serovar, in addition with a high integron prevalence among *Shigella* strains, confirms the propensity of shigellae to acquire and disseminate resistance determinants.

Key words: multi-resistant *Shigella*; integrons; *dfr*

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Introduction

Shigellosis, which is primarily a disease of resource-poor populations, is an important cause of morbidity and mortality among people of all ages living in communities lacking adequate sanitation and safe water. Children under five years old in developing countries are particularly at risk. An estimated 160 million cases and 1.1 million deaths per year are due to shigellosis [1]. *Shigella flexneri* is the most prevalent serotype in Africa [2-4]. Shigellae are noteworthy for their multiple drug resistance, having gradually acquired resistance to most widely used and inexpensive antimicrobial drugs [5-8].

Mobile genetic elements such as plasmids and transposons are involved in the spread of resistance, together with genomic islands and integrons. Integrons are genetic elements that, by site-specific recombination, can integrate gene cassettes, usually antibiotic resistance genes [9]. Class 1 and 2 are the most frequent in Gram-negative bacteria [10]. The structure of class 1 integron includes 5' and 3' conserved segments and a variable region containing gene cassettes. Most previously described class 2 integrons contain the same array of four gene cassettes, three antibiotic resistance gene cassettes (*dfrA1*, *sat* and *aadA1)*, conferring resistance to trimethoprim, streptothricin, and spectinomycin/streptomycin, respectively), and the *orfX* cassette of unknown function. Class 2 integrons have been described in transposon Tn*7* and its derivatives, and their 3' segment contains five *tns* genes involved in transposon movements [11]. The movement of cassettes are catalyzed by site-specific recombination; cassette mobility results in the dissemination of resistance genes.

Integrons have a well-established role in the dissemination of resistance among Gram-

negative pathogens and are thus a useful marker of antibiotic resistance [12]. Trimethoprim is widely used to treat several infectious diseases in Senegal, in combination with sulfonamides. As trimethoprim resistance determinants are often found in gene cassettes, we examined the prevalence of integrons in Senegalese *Shigella* isolates.

Materials and methods

Bacterial isolates

A total of thirty-two *Shigella* strains belonging to serotypes *flexneri* (N = 14), *dysenteriae* (N = 13), and *Shigella boydii* 20 serovar nov. (N = 5) isolated from adults with diarrhoea at a teaching hospital and an urban general hospital in Dakar, Senegal, were included in the study.

Antimicrobial susceptibility

Antibiotic susceptibility was tested with the disk diffusion method on Mueller-Hinton agar (Becton Dickinson Cokeysville, Md), and antibiotics were those used for Enterobacteriaceae, as recommended by the Clinical Laboratories Standards Institute (www.clsi.org).

DNA extraction

Total DNA was extracted with the Qiamp DNA minikit (Qiagen, S.A, Courtaboeuf, France).

Integron detection

The strains were screened by PCR for class 1, 2 and 3 integrons with the primers described above [13,14,15]**.** The 50-µl PCR reaction mix consisted of Taq polymerase buffer, 1.5 mM $MgCl_2$, 200 µM deoxynucleotide triphosphates, 50 pmol of each primer (Isoprim, Toulouse, France), 1 U of Taq, and 25 ng of DNA were amplified in a thermal cycler (Perkin-Elmer 2400, Applied Biosystems). PCR with primers *orf4* (5'-CAAACTATCAGGTCAAGTCTGCTT-3') and *sul1* (5'-GTCCGACATCCACGACGTCTGATC-3') were used to detect the 3' segment usually found in class 1 integrons [15]. PCR amplification of the class 1 integron cassette array used primers 5'CS (5'-GGCATCCAAGCAGCAAG-3') and 3'CS (5'-AAAGCAGACTTGACCTGA-3') [15]. If the 3' segment was absent, amplification was performed with primers located in cassettes selected on the basis of the resistance phenotypes. When a strain yielded two PCR products, the fragments were separated by agarose gel electrophoresis and purified with the QIAquick gel extraction kit (Qiagen, S. A, Courtaboeuf, France). The cassette content of class 2 integrons was characterized with primers located in the cassettes usually found in class 2 integrons: *hep74* (5'-CGGGATCCCGGACGGCATGCACGATTTGT-3'), *aadA3* (5'-GAATGATGTCGTGCACAAC-3') located in the *aadA1* gene cassette (this study), and *orfX2* (5'-AGATACATGATCTTCAGGCC-3') [16].

To characterize the 3' segment of class 2 integrons, amplification was performed using primers *int2CS2* (5'-TACCTGTTCTGCCCGTATCT-3') and *int7S* (5'-TGCCCTGCGTAAGCGGGTGTGGGCGGACA-3') [15].

DNA sequencing

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

Conjugation experiments

Mating experiments were conducted in Luria-Bertani broth with the nalidixic acid-resistant *E. coli* strain C1a as recipient. Transconjugants were selected on Luria-Bertani agar plates containing nalidixic acid (50 µg/ml), and trimethoprim (100 µg/ml) or ampicillin (100 µg/ml). All transconjugants were screened for *intI1* and *intI2* by PCR.

Random amplified polymorphic DNA analysis (RAPD)

RAPD was performed with primers A_4 (5'-TGCCCGGACG-3') and A_5 (5'-GCCGGGCCT-3') [15] (Bioprobe Systems, Montreuil sous bois, France). Strains were considered non-identical if their RAPD patterns differed by at least two bands.

Results

Antibiotic resistance

All the strains were resistant to at least four of the following antibiotics: ampicillin, ticarcillin, tetracycline, trimethoprim, sulfamethoxazole, streptomycin, and chloramphenicol (Table 1).

Int *genes*

Twenty-eight (87.5%) of the 32 strains contained at least one integron, and 26 of these 28 strains contained at least one class 1 integron. The *intI2* gene was detected in 16 strains, either alone (2 strains of *S. flexneri)* or together with *intI1* (14 strains*)*. The *intI1* and *intI2* genes were found together in all *S. boydii* 20 isolates, and in 9 of the 14 *S. dysenteriae* isolates. No class 3 integrons were detected.

Table 1. Characteristics of *Shigella* strains: Resistance phenotypes, cassette arrays, and RAPD profiles

	Resistance phenotypes	5'conserved segment (*intI* genes)	3' segment PCR (kb) (Orf4-Sul1)*/(tns)**	Gene cassettes/RAPD profiles Class 1 integrons Class 2 integrons
S. boydii 20 (N=5)	AmpRTicRSSSRTmpRTeR StrR	*intI1, intI2*	0.7/-	*dfrA5*/5 (F) *dfrA1-sat-aadA1*/5 (F)
S. dysenteriae (N=13)	AmpRTicRSSSRTmpRTeRStrRCmR	*intI1, intI2*	-/- 1.8/-	*oxa30-aadA1*-IS*1*/7 (C) *dfrA1-sat*-orfX/8 (C)
N=9	AmpRTicRSSSRTmpRTeRStrRCmR	-	-	*dfrA1-aadA2/*2 (C) *dfrA1-sat-aadA1*/1(C)
N=2	AmpRSSSRTmpRTeRStrR	*intI1*	1.8/ND	- -
N=2				*dfrA15-aadA1*/2 (C)
S. flexneri (N=14)	AmpRTicRSSSRTmpRTeRStrRCmR	*intI1*	-/ND	*oxa30-aadA1*-IS*1*/6 (E), 1 (D)
N=8				*dfrA15-aadA1*/1 (E)
	SSSRTmpRTeRStrR	*intI1*	0.7/ND	*dfrA15*/2 (E)
N=2	SSSRTmpRTeRStrR	*intI*2	ND/-	
N=2	AmpRTicRSSSRTmpRTeRStrR	-	-	*dfrA1-sat-aadA1*-orfX/2 (E)
N=2				- -

***** class 1 integrons ND: not done
**** class 2 integrons** Amp, ampicillin; Tic, ticarcillin; Te, tetracycline; Str, streptomycin; SSS, sulfamethoxazole, Tmp, trimethoprim; Cm, chloramphenicol

Cassette arrays

In 3' segment-containing class 1 integrons, the gene cassettes arrays were characterized by PCR with primers 5'CS and 3'CS and by sequencing the amplification products. The strains yielded amplicons ranging from 0.7 kb to 1.8 kb. Four different cassette arrays with one (*dfrA5*, *dfrA15*) or two cassettes (*dfrA15-aadA1, dfrA1-aadA2)* were characterized (Table 1). The 14 strains lacking the 3' segment were resistant to streptomycin and spectinomycin, owing to the presence of *aadA* gene cassettes, of which *aadA1* was most prevalent. To determine the cassette arrays of these strains, amplification was performed with primers 5'CS and *aadA3* yielding a PCR product of 1.5 kb. Sequencing of this product showed the presence of two cassettes, *oxa30* and *aadA1*. Dubois *et al*. described *Shigella* strains containing a class 1 integron in which these two cassettes were followed by the insertion sequence IS*1*. Successful amplification with primers AadA3 and is1b (5'-GTGAACGCACTATGGCGACGC TG-3'), located within IS*1* [17], confirmed that the genetic environment was the same as that described by Dubois *et al*. [18].

The class 2 integrons of our strains are heterogenous; two *S. flexneri* strains harbouring exclusively class 2 integrons had a classical organization with the same four cassettes as those found in Tn*7*, *dfrA1*, *sat*, *aadA1* and *orfX***.** Either The *aadA1* or the *orfX* cassette was lacking in all strains containing both class 1 and 2 integrons (Table 1).

Transfer of antibiotic resistance and genetic location of integrons

Resistance to ampicilin, ticarcillin, streptomycin, tetracycline, sulfamethoxazole, trimethoprim, excepted chloramphenicol for one strain of *Shigella dysenteriae* was transferred simultaneously from all *Shigella strains* harbouring class 1 integrons with cassette arrays (*dfrA15*, *dfrA15-aadA1*, *dfrA1-aadA2*). Conjugation experiments were unsuccessful for strains harbouring unusual class 1 and 2 integrons supporting the chromosomal location of these integrons. Strains harbouring the classical class 1 and 2 integrons yielded transconjugants on trimethoprim suggesting their plasmidic location.

Distribution of integrons among RAPD types and serotypes

Six RAPD profiles were identified, three in *Shigella dysenteriae* (A, B, C), two in *Shigella flexneri* (D, E), and one in *Shigella boydii* 20 (F) (Table 1). Two *Shigella dysenteriae* strains

belonging to the B profile did not contain integrons. All *Shigella dysenteriae* and the majority of *Shigella flexneri* strains (11/12) harbouring integrons showed a unique RAPD profile but had a different integron carriage except *Shigella boydii* 20 strains which showed a unique profile with the same integron content (Table 1). These data revealed a clonal spread of integrons among serotypes and a transfer of integrons between different serotypes.

Discussion

Antibiotic resistance is common in *Shigella spp*. [7,19,4,20]. In Senegal, multiple resistance to ampicillin, sulfamethoxazole, trimethoprim and tetracycline is related to the intensive first-line use of these antibiotics to treat diarrhoeal illnesses and other infectious diseases. Here we found that 87.5% of 32 *Shigella* strains isolated in Senegal contained at least one integron. This marked dissemination of integrons among *Shigella spp* is partly linked to the propensity of this genus to acquire plasmids, as multidrug resistance integrons are usually plasmid-borne [18]. In this study, class 1 integrons were the most prevalent, in keeping with the results of other studies of African *Shigella* isolates [18]. In contrast, integron prevalence is very low in other parts of the world [20,21,22]. We found class 2 integrons in half the 32 strains studied here. Other studies have shown that class 2 integrons are more prevalent in industrialized and/or emerging countries [23,24,25]. Among shigellae, class 2 integrons tend to be associated with *Shigella sonnei* [26,20,27,28]. We detected class 1 and 2 integrons in *S. dysenteriae*, *S. flexneri* and in the new serovar of *S. boydii* 20. The latter was first isolated in Canada from patients who had recently travelled to Cuba, Ethiopia, India, Guatemala or Mexico [28]. In Senegal, this serovar was identified between July and September 2000.

Class 1 integrons were highly diverse, with five different integrons harbouring a *dfr* cassette, alone or associated with *aadA1*. Furthermore, all class 2 integrons detected contained the *dfrA1* cassette and 8 out of 16 also contained *aadA1* cassette. Our results thus showed that trimethoprim and streptomycin/spectimonycin resistant gene cassettes were highly encountered in Senegalese *Shigella* isolates. In sub-Saharan Africa, trimethoprim is widely used, in combination with sulfonamides, to treat diarrhoeal illnesses and urinary tract infections as well as to prevent opportunistic infections in HIV-infected and malarial patients [29,30]. Streptomycin is intensively used to treat tuberculosis, in combination with other drugs. The high prevalence of *dfr* and *aadA1* gene cassettes in integron-containing *Shigella* strains may thus be related to the use of these antibiotics. However, the *dfr* and *aadA* cassettes are also very common in integrons harboured by other *Enterobacteriaceae* species isolated from patients in industrialized countries [20,21]. In a previous study in Senegal we also found a high prevalence of *dfr* and *aadA* cassettes in integron-containing enteroaggregative and enteroinvasive *Escherichia coli* (*E. coli*) strains [31]. Horizontal transfer could readily occur between *E. coli* and *Shigella*, which are both enteric pathogens. Indeed, we successfully obtained transconjugants with *dfr*-containing strains. These two studies showing a high prevalence of *dfr*-containing integrons in enteric pathogens strongly challenge the use of trimethoprim in Senegal.

We detected the unusual class 1 integron with partial or total deletion of the 3' segment, as previously described in *Shigella dysenteriae* and *Shigella flexneri* [26,18]. In our strains this atypical class 1 integron was found either alone or associated with a class 2 integron lacking the *aadA1* or *orfX* cassette. This type of integron was associated to multiple resistance (as shown in Table 1) and confirm the role of integron in antibiotic-resistance. Otherwise, the deletion of the *aadA1* cassette could result from intI1 integrase-catalyse co-integrate formation between a class 1 and 2 integron or a possible RecA-dependent homologous recombination between two copies of the same cassette in both classes of integron [32].

Integron carriage was unrelated to the RAPD profile in *Shigella dysenteriae* and *Shigella flexneri*, whereas *Shigella boydii* 20 strains had a unique profile with the same integron content, indicating clonal spread. Previous studies also found similar patterns for *S. boydii* serotype 20 by using pulsed-field gel electrophoresis and ribotyping, and inferred that this serotype could be homogeneous [28,33].

The detection of integrons in a new *Shigella* serovar, in addition with high integron prevalence among *Shigella* strains, confirms the propensity of shigellae to acquire and disseminate resistance determinants.

The presence of integrons in *Shigella* may have important clinical implications, as multiple gene cassettes could be captured by such strains easily leading to multidrug resistance, even to broad-spectrum antibiotics such as third-generation cephalosporins and quinolones.

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References

1. Kotloff K, Winickoff JP, Ivanoff JB, Clemens JD, Swerdlow DL, Sansonnetti PJ, Adak GK, and Levine MM (1999) Global burden of *Shigella* infections: implications for vaccine development and implementation of controls strategies. Bull WHO 77: 651-66.
2. Bonfiglio G, Simporé J, Pignaletti S, Musumeci S, Solinas ML (2002) Epidemiology of bacterial resistance in gastro-intestinal-pathogens in a tropical area. Int J Antimicrob Agents 20: 387-89.
3. Iwalokun BA, Gbenle GO, Smith SI, Ogunledun A, Akinsinde KA, and Omonigbehin EA (2001) Epidemiology of shigellosis in Lagos, Nigeria: Trends in antimicrobial resistance? J Health Popul Nutr 19: 183-90.
4. Shapiro R, Kuma L, Phillips-Howard P,Wells JG, Adcock P, Brooks J, Ackers, ML, Ochieng JB, Mintz E, Wahlquist S, Waiyaki P, and Slutsker L (2001) Antimicrobial resistance bacterial diarrhea in rural western Kenya. J Infect Dis 183: 1701-04.
5. Vila J, Gascon J, Abdalla S, Marco F, Moreno A, Corachan M, and De Anta TJ (1994) Antimicrobial resistance of *Shigella* isolates causing traveler's diarrhea. Antimicrob Agents Chemother 38: 2668-70.
6. Lima AAM, Lima VL, Pinho MC, Barros EA, Teixeira MJ, Martines MCV, and Guerrant RL (1995) High frequency of strains multiply resistant to ampicillin, chloramphenicol, and tetracycline, isolated from patients with shigellosis in northeastern Brazil during the period 1988-1993.Antimicrob Agents Chemother 39: 256-59.
7. Bogaerts J, Verhaegen J, Munyabikali JP, Mukantabana B, Lemmens P, Vandeven J, Vandepitte J (1997) Antimicrobial resistance and serotypes of *Shigella* isolates in Kigali, Rwanda (1983-1993): Increasing frequency of multiple resistance Diag Microbiol Infect Dis 28: 165-71.
8. Chu YW, Houang ETB, Lyon DJ, Ling, JM, NG, TK, and Cheng AFB (1998) Antimicrobial resistance of S*higella flexneri*, and *Shigella sonnei* in Hong Kong, 1986 to 1995. Antimicrob Agents Chemother 42: 440-43.
9. Hall RM, and Collis CM (1998) Antibiotic resistance in gram negative-bacteria: the role of gene cassettes and integrons. Drug Resist updates 1: 109-119.
10. Goldstein C, Lee MD, Sanchez S, Hudson C, Phillips B, Register B, Grady M, Liebert C, Summers AO, White DG, and Maurer JJ (2001) Incidence of Class 1 and 2 Integrases in Clinical and Commensal Bacteria from Livestock, Companion Animals, and Exotics. Antimicrob Agents Chemother 45: 723-26.
11. Flores C, Qadri MI, and Lichtenstein C (1990) DNA sequence analysis of five genes; *tnsA, B, C, D* and E, required for Tn*7* transposition. Nucleic Acids Research 18: 901-11.
12. Fluit AC, Schmitz FJ (2004) Resistance integrons and super-integrons. Clin Microbiol Infect 10: 272-88.
13. Bissonette L, Roy PH 1992. Characterization of In0 of *Pseudomonas aeruginosa* plasmid pVS1, an ancestor of integrons of multiresistant plasmids and transposons of gram negative bacteria. J Bacteriol 174: 1248-57
14. Mazel D (2004) Integron and the origin of antibiotic resistance gene cassettes. ASM News 70: 520-25.
15. Ploy MC, Denis F, Courvalin P, and Lambert T (2000) Molecular characterization of integrons in *Acinetobacter baumanii*: Description of an hybrid class 2 integron. Antimicrob Agents Chemother 44: 2684-88.
16. White P, McIver J, Rawlinson WD (2001) Integrons and gene cassettes in *Enterobacteriaceae*. Antimicrob Agents Chemother 45: 2658-61.
17. Ohtsubo H, and Ohtsubo E (1978) Nucleotide sequence of an insertion element, IS*1*. Proc. Natl. Acad. Sci. USA. **75**: 615-19.
18. Dubois V, Parizano MP, Arpin C, Coulange L, Bezian MC, Quentin C (2007) High genetic stability of integrons in clinical isolates of *Shigella spp*. of worldwide origin. Antimicrob Agents Chemother 51: 1333-40.
19. Navia MM, Capitano J, Ruiz J Vargas M, Urassa H, Schellemberg, D, Gascon J, and Vila J (1999) Typing and characterization of mechanisms of resistance of *Shigella spp* isolated from feces of children under 5 years of age from Ifakara, Tanzania. J Clin Microbiol 37: 3113-17.
20. Oh J, Yu HS, Kim SK, Seol SY, Cho DT, and Lee JC (2003) Changes in patterns of antimicrobial susceptibility and integron carriage among *Shigella sonnei* isolates from southwestern Korea during epidemic periods. J Clin Microbiol 41: 412-13.
21. Delappe N, O'Halloran F, Fanning S, Corbett-Feney G, Cheasty T, and Cormican M (2003) Antimicrobial resistance and genetic diversity of *Shigella sonnei* isolates from Western Ireland, an area of low incidence of infection. J Clin Microbiol 41: 1919-24.
22. Navia MM, Ruiz J, and Vila J (2004) Molecular characterization of the integrons in *Shigella* strains isolated from patients with traveler's diarrhea. Diag Microbiol Infect Dis 48: 175-79.
23. Ahmed AM, Nakano H, and Shimamoto T (2005) Molecular characterization of integrons in non- typhoid *Salmonella* serovars isolated in Japan: description of an unusual class 2 integron. J Antimicrob Chemother 55: 371-74.
24. Ranjbar R, Aleo A, Giammanco GM, Dionisi AM, Sadeghifard N, and Mammina C (2007) Genetic relatedness among isolates of *Shigella sonnei* carrying class 2 integrons in Tehran, Iran, 2002-2003. BMC Infect Dis 7: 62.
25. McIver C, White PA, Jones LA, Karagiannis T, Harkness J, Mariott D, and Rawlinson WD (2002) Epidemic strains of *Shigella sonnei* biotype g carring integrons. J Clin Microbiol 40: 1538-40.
26. Pan JC, Ye R, Meng DM, Zhang W, Wang HQ, Liu KZ (2006) Molecular characteristics of class 1 and 2 integrons and their relationships to antibiotic resistance in clinical isolates of *Shigella sonnei* and *Shigella flexneri*. J Antimicrob Chemother 58: 288-96.
27. Gassama Sow A, Diallo MH, Boye CS, Garin B, Sire JM, Sow AI, Aïdara-Kane A (2006) Class 2 integron-associated antibiotic resistance in *Shigella sonnei* isolates in Dakar, Senegal. Int J Antimicrob Agents 27: 267-70.
28. Woodward DL, Clark CG, Caldeira RA, Ahmed R, Soule G, Bryden L, Tabor H, Melito P, Foster R, Walsh J, Ng LK, Malcolm GB, Strockbine N, Rodgers FG (2005) Identification and characterization of *Shigella boydii* 20 serovar nov., a new and emerging *Shigella* serotype. J Med Microbiol 54: 741-48.
29. Van Oosterhout JJ, Laufer MK, Graham SM, Thumba F, Perez MA, Chimbiya, N, Wilson L, Chagomerana M, Molyneux ME, Zijlistra EE, Taylor TE, Plowe CV (2005) A community-based study of the incidence of trimethoprime-sulfamethoxazole-preventable infections in Malawians adults living with HIV 2. J Acquir Immun Defic Syndr 39: 626-31.
30. Thera MA, Sehdev PS, Coulibaly D, Traore K, Garba MN, Cissoko Y, Kone A, Guindo A, Dicko A, Beavogui AH, Djimbe AA, Lyke KE, Diallo DA, Doumbo, OK, Plowe CV (2005) Impact of trimethoprim-sulfamethoxazole prophylaxis on falciparum malaria infection and disease. J Infect Dis 192: 1823-29.

31. Gassama A, Aïdara-Kane A, Chainier D, Denis F, Ploy MC (2004) Integron-associated antibiotic resistance in enteroaggregative and enteroinvasive *Escherichia coli*. Microb Drug Resist 10: 27-30.
32. Gassama Sow A, Diallo MH, Gatet M, Denis F, Kane-Aïdara A, and Ploy MC (2008) Description of an unusual class 2 integron in *Shigella sonnei* isolates in Senegal (sub-Saharan Africa). J Antimicrob Chemother 62: 843-51.
33. Grimont F, Lejay-Collin M, Talukder KA, Carle I, Issenhuth S, Leroux K and Grimont PAD (2007) Identification of a group of shigella-like isolates as *Shigella boydii* 20. J Med Microbiol 56: 749-54.

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