IS6100-mediated genetic rearrangement within the complex class 1 integron In104 of the Salmonella genomic island 1

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Sir, Multidrug-resistant Salmonella enterica serovar Typhimurium DT104 (where DT stands for definitive type) emerged during the 1980s as a cause of many outbreaks in humans and animals, most isolates exhibiting resistance to a core group of antimicrobials including ampicillin/amoxicillin, chloramphenicol/florfenicol, streptomycin/spectinomycin, sulphonamides and tetracyclines (ACSSuT phenotype).1 The genes responsible were shown to be located on a 13 kb multidrug resistance region (MDR region) that constitutes a complex class 1 integron. This cluster, recently named In104, belongs to the integron In4 nomenclature.1 Several variants of SG1 have also been described over time in a wide variety of S. enterica serovars and in Proteus mirabilis.

In this study, we examined an S. enterica serovar Typhimurium strain 72-21880-11 isolated from the faeces of a healthy cow in Denmark in 2000, which displayed resistance to chloramphenicol/florfenicol, streptomycin/spectinomycin and tetracycline. To assess whether this strain harbours SG1, PCRs were performed using primers corresponding to the left and right junctions of SG1 in the Salmonella chromosome as described previously.2 PCR results were positive for the left junction with the chromosomal thdF gene and for the right junction with the int2 gene of the retron sequence specifically found downstream of SG1 in the serovar Typhimurium, indicating that this strain contained SG1 at the same location as in other isolates.2 To confirm the presence of the entire SG1, PCR mapping of the 5’ region of SG1 (the first 10 kb) was performed as described previously.1 All of the PCR results were positive. The presence of the remaining non-MDR region of SG1 was assessed by Southern blot hybridization of XbaI-digested genomic DNA with the p1-9 probe as previously described.2 The Southern blot profile of the serovar strain 72-21880-11 was similar to that obtained for the control strain of serovar Typhimurium DT104 harbouring SG1 with two XbaI fragments of 4 and 9 kb (data not shown). To confirm the location of the MDR region within SG1, PCRs were performed using primers SO26-FW/int-RV and DB-T1/MDR-B2 coding to the boundaries of the integron with the SG1 backbone. The two PCRs gave positive results, indicating that the MDR region was at the normal position within the SG1 backbone, i.e. between the res gene and the open reading frame S044.

The unusual resistance pattern of the strain 72-21880-11 suggests that genetic variations occurred within the In104 complex integron, which were assessed by PCR mapping of the antibiotic resistance gene cluster as described previously3 (Figure 1a). All PCR products were obtained except the one corresponding to fragment E specific for the bla_PSE-1 gene cassette. This result was confirmed by the cassette-array PCR for which only one fragment of 1 kb was obtained, corresponding to the aadA2 gene cassette. Additional PCRs were also carried out, indicating that the variant region is located downstream of orf2 (data not shown). The region between groEL/int1 and IS6100 was then amplified by PCR and the 1.2 kb product sequenced by Beckman Coulter Genomics (Takeley, UK). The nucleotide sequence obtained (deposited in GenBank under accession number GU830872) allowed us to resolve the genetic organization of the variant antibiotic resistance gene cluster, which consisted of the groEL/int1 gene, followed by only 403 bp of the bla_PSE-1 gene and by the IS6100 element (Figure 1a). To confirm this genetic organization, we performed a Southern blot hybridization of genomic DNA cut either by HindIII or by XbaI and XhoI, and using as a probe the XbaI fragment of the antibiotic resistance gene cluster, which consisted of the groEL/int1 gene, followed by only 403 bp of the bla_PSE-1 gene and by the IS6100 element (Figure 1b). The sizes of the bands correlated with the genetic organization characterized previously by PCRs and sequencing. The cluster described in this study constitutes a new SG1 variant and we propose to name it SG1-T according to the nomenclature.

The genetic structure described here could be explained by an intramolecular transposition of the IS6100 element leading to the insertion of a second IS6100 copy within bla_PSE-1. Subsequently, a single cross-over event between the two IS6100 elements in the same orientation would have led to the deletion of the central region including a part of the retron pSTF3, comprising nearly the entire In104 complex class 1 integron.2 Southern blot profiles of the studied strain were clearly distinct from those of the control strain harbouring the classic SG1 (Figure 1b). The sizes of the bands correlated with the genetic organization characterized previously by PCRs and sequencing. The cluster described in this study constitutes a new SG1 variant and we propose to name it SG1-T according to the nomenclature.

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Transparency declarations

None to declare.

References

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Persistent isolation of *Salmonella* Concord harbouring CTX-M-15, SHV-12 and QnrA1 in an asymptomatic adopted Ethiopian child in Spain also colonized with CTX-M-14- and QnrB-producing Enterobacteriaceae

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Sir,

Endemicity of extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae in orphanages has been reported in different developing countries where children and caregivers are colonized with these type of isolates.1 ESBLs in salmonellae are increasing in prevalence, with the propensity to carry more than one ESBL within or without other transmissible resistance mechanisms. We report the persistent recovery of CTX-M-15- and SHV-12-carrying *Salmonella enterica* serotype Concord isolates also harbouring QnrA1 from the stool cultures of an Ethiopian child in Madrid. The 1-year-old boy, transiently adopted in December 2008 through a non-governmental organization, came from an orphanage in Addis Ababa, Ethiopia, and was immediately admitted to the Paediatric Intensive Care Unit of the Hospital Universitario Ramón y Cajal in Madrid (Spain). Prior to his arrival and due to a febrile syndrome, he had been sequentially treated with standard doses of ceftriaxone, piperacillin/tazobactam and amoxicillin/clavulanate; the latter was suspended due to persistent diarrhoea. Once in Spain, although it was not microbiologically documented, he was clinically diagnosed with urinary sepsis and acute obstructive renal failure. The patient stayed in an intensive care unit for 7 days until surgery to correct an obstructive uropathy. He received meropenem and fluconazole for 19 days. After hospital discharge, oral amoxicillin/clavulanate was administered.

A routine stool culture submitted at admission rendered the isolation of an ESBL-producing *Salmonella* Concord isolate (S1). Due to the resistance pattern and the infrequent serotype in our country, subsequent stool samples were requested. In addition to standard stool culture plating, the chromogenic agar medium chromID ESBL (bioMérieux, Marcy l’Étoile, France) was used. Intrafamilial faecal carriage of ESBL-producing Enterobacteriaceae was also screened during January–March 2009. The CTX-M-15-producing *Salmonella* Concord strain 3728 and its *Escherichia coli* (JS3 AzI) transconjugant were used as controls.2,3 Molecular methods were performed as previously described.4–7 Three additional ESBL-producing *Salmonella* Concord isolates (S2–S4) as well as three ESBL-producing *Escherichia coli* (E1–E3) and three ESBL-producing *Klebsiella pneumoniae* (K1–K3) isolates were recovered monthly (January–March 2009) from the patient. We cannot rule out the potential acquisition of the ESBL-producing *E. coli* and *K. pneumoniae* isolates after the child’s arrival in Spain as the search for these isolates was not performed for the first faecal culture. The four *Salmonella* Concord isolates were resistant to all β-lactams except cefotaxin and carbapenems. Cefotaxime, ceftazidime and cefepime MICs (standard microdilution) were >256 mg/L, while those of the combinations cefotaxime/clavulanate and ceftazidime/clavulanate (fixed clavulanate concentration of 4 mg/L) were 1 and 2 mg/L, respectively. These isolates simultaneously produced a CTX-M-15 and an SHV-12 ESBL. Moreover, they were resistant to nalidixic acid (MIC ≥32 mg/L) with a ciprofloxacin MIC of 0.25 mg/L. All isolates were resistant to gentamicin and tobramycin and susceptible to kanamycin, amikacin and netilmicin. They were also resistant to trimethoprim, sulphonamides, tetracycline and chloramphenicol, but susceptible to tigecycline (Table 1).

The first recovered *Salmonella* isolate (S1) harboured three plasmids of ~50, 100 and 340 kb. Both bla<sub>CTX-M-15</sub> and bla<sub>SHV-12</sub> genes were demonstrated to be located in the latter non-conjugative plasmid of incompatibility group IncHI2 by hybridization studies. The other three *Salmonella* isolates contained at least three plasmids ranging from ~50 to 250 kb, but did not harbour the 340 kb plasmid, as did S1 [Figure S1, available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/)]. The location of both bla<sub>CTX-M-15</sub> and bla<sub>SHV-12</sub> was also demonstrated by hybridization and genes were found on the IncHI2 non-conjugative plasmid of 250 kb. It is of note that the four *Salmonella* isolates belonged to the same clone as the PFGE patterns were indistinguishable (Table 1) and presented high similarity (two bands of difference) to an isolate previously recovered in France from the stools of an adopted child who had come from Ethiopia.2 Serotype Concord is very unusual in Spain and, during the last 5 years (2004–08), only three serotype Concord isolates (0.01%) were identified in the Spanish Reference *Salmonella* Laboratory (National Reference Centre, Majadahonda, Spain) and none of them was an ESBL producer (A. Echeita, National Reference Centre, personal communication).

Two out of the three *E. coli* isolates were genetically related (E1 and E3). The three isolates produced a CTX-M-14 enzyme and one of them (E2) was also positive for qnrB4. In all cases, bla<sub>K_{E1-13}</sub> was detected on an ~120 kb conjugative plasmid. Two of these plasmids belonged to the IncF group, and the other one was non-typeable (Table 1). The three ESBL-producing *K. pneumoniae* isolates presented highly related PFGE patterns...