Characterization of ESBL-producing *Shigella sonnei* in Spain: expanding the geographic distribution of clone ST152/CTX-M-27

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Abstract

We describe the first occurrence in Spain of community cases of CTX-M-27-producing *Shigella sonnei* ST152 resistant to quinolones and azithromycin. Cases included adult males, but also one paediatric case. The isolates were clustered together with an Australian isolate and differed from other outbreak-causing strains in England by more than 50 alleles. They carried the $bla_{\text{CTX-M-27}}$ gene on an 83 Kb F2:A-:B plasmid, similar to that found in a British isolate.

Shigella sonnei is the leading species of shigellosis in high-income countries (1). The first case of ESBL-producing *Shigella* was documented in 2004 and the recent emergence of ESBL-producing isolates has also been reported (1). Tracing the dissemination of these isolates is important because therapeutic options are limited, since they are resistant to first-line (ciprofloxacin and azithromycin) and second-line options (third-generation cephalosporins). Increased use of whole-genome sequencing characterization of multidrug-resistant isolates, together with the availability of public genome repositories means that it is possible to establish links that would otherwise go unnoticed.

Although there are no studies available on the prevalence of ESBL in *Shigella sonnei*, ST152 has been widely reported among ESBL-producing isolates, mainly CTX-M-3- and CTX-M-15-producing (2). More recently, CTX-M-27-producing *S. sonnei* isolates belonging to ST152 have been reported in 4 cases in the United Kingdom, in 2015 (3), in 20 cases in Australia between 2019 and 2020 (4), and in 1 case in Switzerland in 2019 (2), with men who have sex with men (MSM) being identified as the primary risk factor in most cases.

In August 2020, a 22-year-old man with a medical history of unprotected MSM relationships was admitted to hospital with infectious colitis, and faeces culture yielded *S. sonnei*. Five weeks later, a 37-year-old HIV-positive man was admitted with dysentery symptoms and a faeces sample tested positive for *S. sonnei*. One year later, four more cases were diagnosed in our area, of which two were adult males, one case was a 5-year-old child, and the fourth was female. Of these 2021 cases, only one required hospital admission.

Initial identification of bacterial isolates was by MALDI-TOF and MICs were obtained with Microscan MDMR1 panels. MICs for azithromycin and

ciprofloxacin were obtained using gradient strips. All isolates were resistant to third-generation cephalosporins, co-trimoxazole, azithromycin (>=256 mg/l) and ciprofloxacin (8 mg/l) and a positive ESBL phenotype was detected by the double-disc method (cephalosporin with and without clavulanic acid). No epidemiological link or recent history of travel abroad could be established, and, except for the first case, the others had no history of MSM.

All isolates were sequenced on an Illumina platform (MiSeq), submitted to NCBI (BioProject ID: PRJNA691710), followed by assembly with CLC Genomics software (Accession No. PRJNA691710). The assemblies were analysed using the tools of the Center for Genomic Epidemiology (CGE; https://cge.cbs.dtu.dk/services/)(5). All isolates were assigned to the ST152 clone. Our strains harboured IncFII (F2:A-:B-), IncB/O/K/Z, Col(MD18), Col(pHAD28) and Col(BS5R) plasmids, and carried *bla*$_{\text{CTX-M-27}}$, *mph*(A), *ermB*, *dfrA*17, *sul1*, *sul2*, *qnrB19, bla*$_{\text{TEM-1}}$ and *aph(6)-Id* resistance determinants, and three mutations in the quinolone resistance-determining region (QRDR) (in *gyrA* S83L and D87G and *parC* S80I). All resistance determinants were shared by most of the previously reported CTX-M-27-producing *S. sonnei* (2–4) except for *qnrB19, sul1* and *dfrA17*, which were found only in some isolates.

The first isolate (20200414) selected was sequenced with MinION technology (Oxford Nanopore Technologies Inc., Oxford, UK). Library preparation was performed with the Rapid Barcoding Kit (SQK-RBK004) and raw signals stored in Fast5 file format were basecalled using MinKNOW ONT software (v1.4.2) (filtering criteria: length >1000, quality >7). Canu v.1.8 was used for assembly and Unicycler v.4.8 for hybrid genome assemblies generated from MiSeq and MinION data.(6) Plasmid replicons were then analysed with the PlasmidFinder

tool (https://cge.cbs.dtu.dk/services/PlasmidFinder/). which confirmed that the *bla*$_{CTX-M-27}$ gene was carried on an 83 kb plasmid with the formula F2:A-:B-, which matched that found in the Swiss (Accession No. CP049168) (2) and English (Accession No. KX008967) (3) isolates, which were the only ones found to be similar after a Basic Local Alignment Search Tool (BLAST) search. The BLAST Ring Image Generator (BRIG) (7) was used to compare the three plasmids (Figure 2). Our plasmid had 92% coverage, mean identity of >99%, and lacked the *bla*$_{TEM-1}$ region when compared with the 88 kb English plasmid, and 79% coverage and mean identity of 99.9% with the 69 kb Swiss plasmid.

The close genetic environment of *bla*$_{CTX-M-27}$ in our strains, annotated with the Rapid Annotations using Subsystems Technology (RAST) server (http://rast.nmpdr.org/) and ISFinder (https://www-is.biotoul.fr), were compared with those of previously reported isolates (2–4) and one that was found by searching Enterobase (one isolate from the USA, Accession No. SRS2523050). Two genetic structures have been detected in all previously reported CTX-M-27-producing *S. sonnei* (2–4): an IS*903B*-*bla*$_{CTX-M-27}$-IS*Ecp1* unit and an IS903-*bla*$_{CTX-M-27}$-IS*15DIV* unit (Figure 1), which suggests that the acquisition of the *bla*$_{CTX-M-27}$ genes occurred in at least two separate events. Our strains lacked IS*Ecp1*, and IS*26* was found upstream of the *bla*$_{CTX-M-27}$ unit. This structure had identical surroundings to those found in all the English isolates (3), except that our isolates were highly resistant to quinolones.

A core genome comparison was performed using EnteroBase's *Escherichia coli/Shigella* database (http://enterobase.warwick.ac.uk/species/ecoli), based on allelic differences found with the loci core genome scheme (<5 alleles of difference were considered related) (8), as well as a SNPs analysis of the whole

genome present in 95% of the compared genomes and using the first isolate (20200414) of our region as reference. The comparison included previously reported CTX-M-27-producing *S. sonnei* isolates (2–4): 2 isolates from the English outbreak (Accession No. SRS1439910 and SRS1439888), 8 from the Australian outbreak (including representatives of two different branches, Accession No: SRS5701367, SRS6935867, SRS6935850, SRS6935848, SRS6935845, SRS6935843, SRS6935834, SRS6935821), 2 non-outbreak isolates (the USA isolate, the Swiss isolate, SAMN14133045) and the Spanish isolates. Allele 2 of IncFII was detected in all of them, except in the isolate from the USA and one of the Australian isolates. A BLAST comparison of FII_2-positive CTX-M-27-producing *S. sonnei* genomes with our plasmid yielded a mean 77 kb similarity with our 83 kb plasmid, and a mean homology of 99.6%.

This analysis revealed that the Spanish isolates and one Australian isolate, which also harboured 3 QRDR mutations and *qnrB1*, *sul1* and *dfrA17* genes, were closely clustered (less than 5 alleles/14-37 SNPs of difference). This cluster differed by more than 50 alleles from the English outbreak and the main group in the Australian outbreak.

This is the first description of ESBL-producing *S. sonnei* in Spain. Based on our results, the Spanish isolates are related to strains from two other continents that showed a similar resistome and genetic platform containing the *bla* genes, suggesting common acquisition and rapid spread. Although cases in adult men with a history of sexual transmission have been described in other countries (9), our cluster also includes a paediatric patient and a woman within two months of each other. This is evidence of transmission to secondary cases in the

community, which raises concerns about limited therapeutic options if a vulnerable population is affected.

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Transparency declarations

None to declare.

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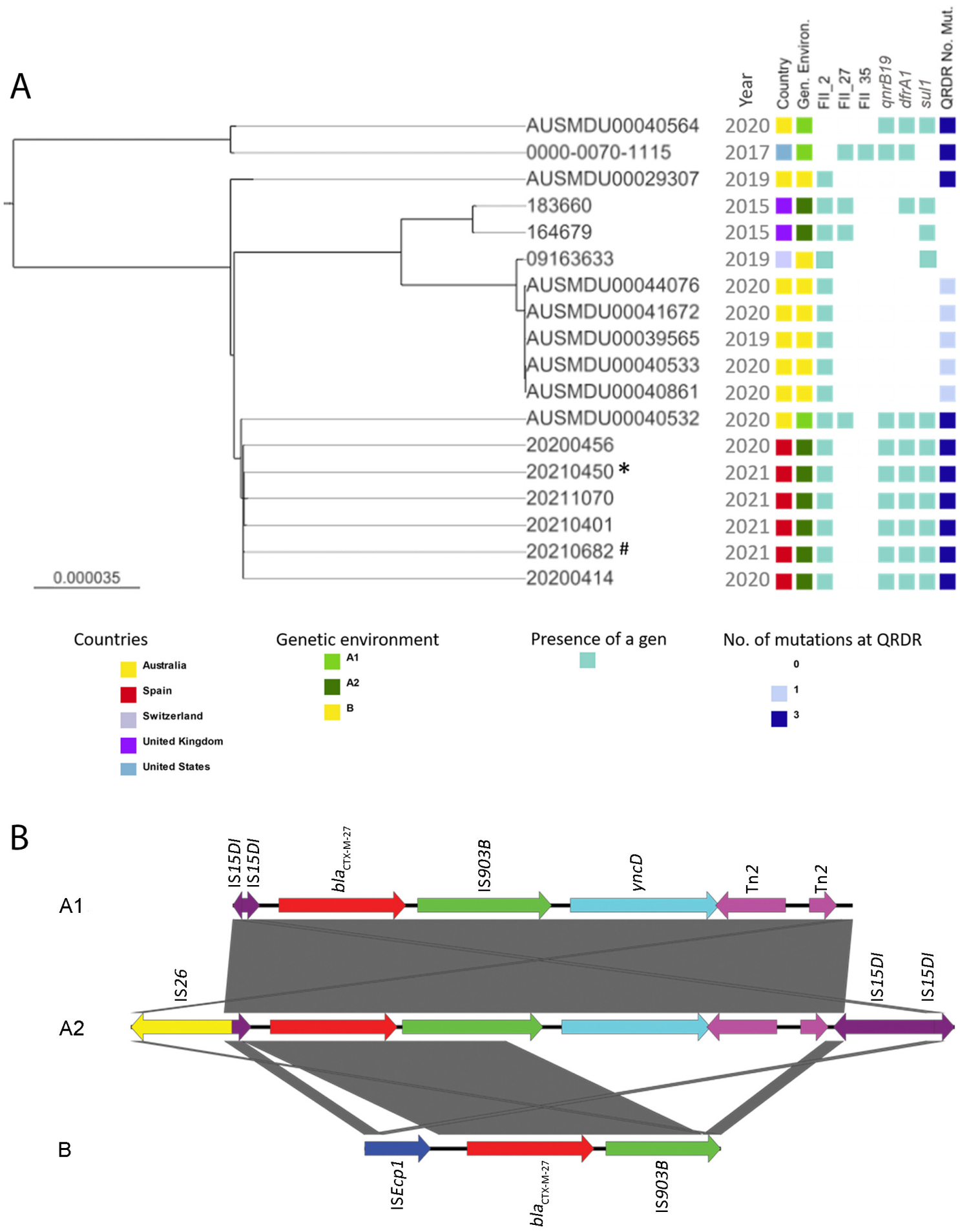
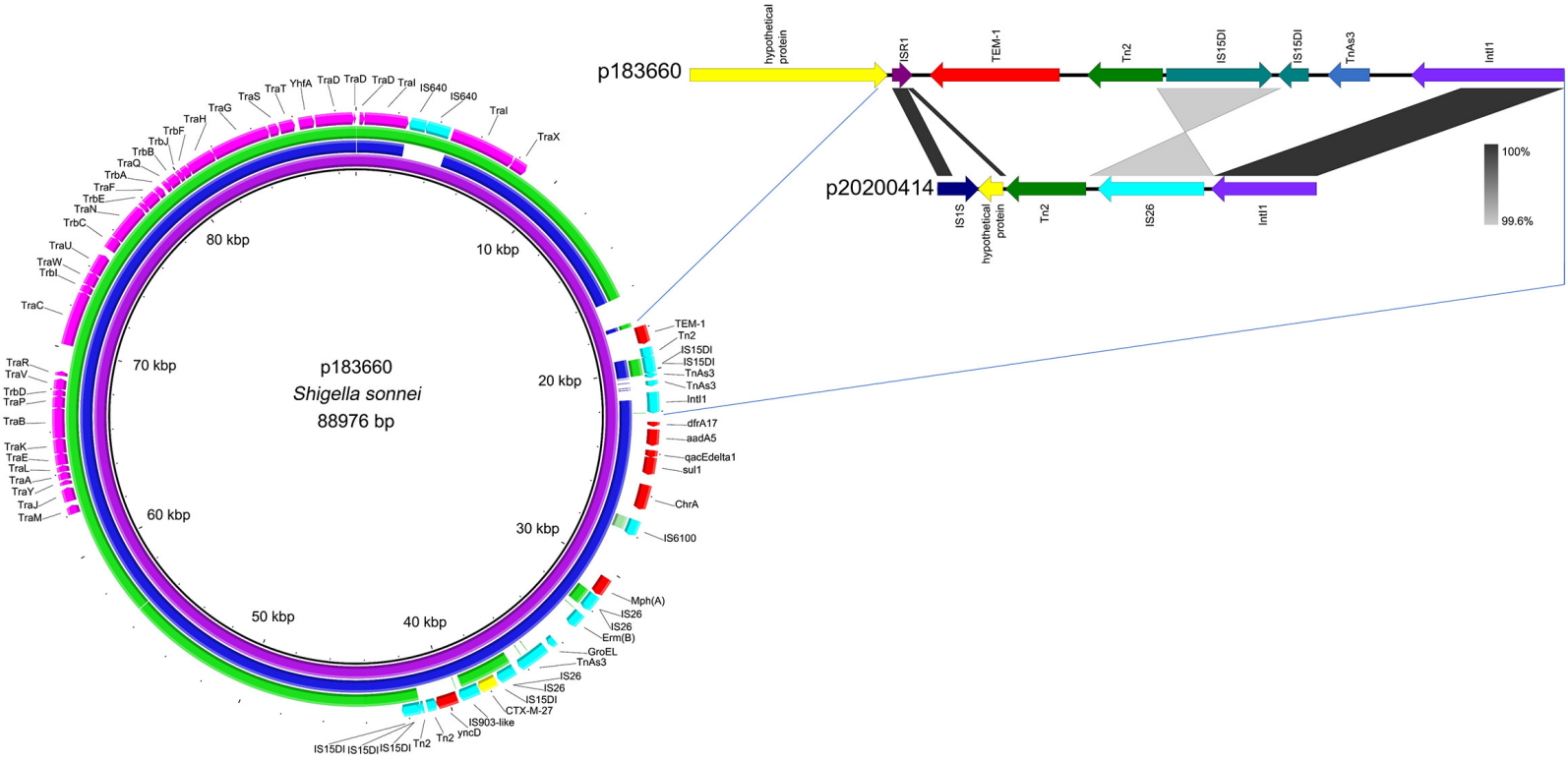
Figure 1. A) Core-genome dendrogram of 18 CTX-M-27-producing *S. sonnei* isolates, inferred using the SNP tree tool based on the cgMLST V1 + Hierarchical Clustering (HierCC) V1 scheme from EnteroBase. Tips are coloured according to year and country of detection, the presence of different resistance determinants, the number of mutations in the quinolone resistance-determining region (QRDR) and the genetic environment surrounding CTX-M-27 (Gen. E), * isolate from the paediatric case and # isolate from the female case. B) EasyFig v2.2.5 was used to represent the $bla_{\text{CTX-M-27}}$-containing genetic environments compared in this study. The genetic structures were arbitrarily named A and B; type A was divided into A1 and A2 due to the presence of an additional IS*26* sequence.

Figure 2. BRIG comparison of p183660 (purple ring) and p0916363 (green ring) with the long-read sequenced $bla_{\text{CTX-M-27}}$-carrying plasmid p20200414 (blue ring) analysed in this study. The arrangement of $bla_{\text{CTX-M-27}}$ is highlighted by the yellow arrow, that of the antibiotic or metal resistance determinants by red arrows, while the insertion sequences are highlighted in light blue. The TEM-1 environment presents in p183660 and the differences found in p20200414 are showed in the extended right image.


A
Year
Country
Gen. Environ.
FII_2
FII_27
FII 35
qnrB19
dfrA1
sul1
QRDR No. Mut.
AUSMDU00040564 2020
0000-0070-1115 2017
AUSMDU00029307 2019
183660 2015
164679 2015
09163633 2019
AUSMDU00044076 2020
AUSMDU00041672 2020
AUSMDU00039565 2019
AUSMDU00040533 2020
AUSMDU00040861 2020
AUSMDU00040532 2020
20200456 2020
20210450 * 2021
20211070 2021
20210401 2021
20210682 # 2021
20200414 2020
0.000035
Countries
Australia
Spain
Switzerland
United Kingdom
United States
Genetic environment
A1
A2
B
Presence of a gen
No. of mutations at QRDR
0
1
3
B
A1
A2
B
IS15DI
IS15DI
bla CTX-M-27
IS903B
yncD
Tn2
Tn2
IS26
IS15DI
IS15DI
ISEcp1
bla CTX-M-27
IS903B


p183660
Shigella sonnei
88976 bp
10 kbp
20 kbp
30 kbp
40 kbp
50 kbp
60 kbp
70 kbp
80 kbp
TraX
TraI
IS640
IS640
TraD
TraD
TraD
TraI
YhfA
TraT
TraS
TraG
TraH
TrbF
TrbJ
TrbB
TraQ
TrbA
TraF
TrbE
TraN
TrbC
TraU
TraW
TrbI
TraC
TraR
TraV
TrbD
TraP
TraB
TraK
TraE
TraL
TraA
TraY
TraJ
TraM
TEM-1
Tn2
IS15DI
IS15DI
TnAs3
TnAs3
IntI1
dfrA17
aadA5
qacEdelta1
sul1
ChrA
IS6100
Mph(A)
IS26
IS26
Erm(B)
GroEL
TnAs3
IS26
IS26
IS15DI
CTX-M-27
IS903-like
yncD
Tn2
Tn2
IS15DI
IS15DI
IS15DI
p183660
hypothetical protein
ISR1
TEM-1
Tn2
IS15DI
IS15DI
TnAs3
IntI1
p20200414
IS1S
hypothetical protein
Tn2
IS26
IntI1
100%
99.6%