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Increasing trend of antimicrobial resistance in *Shigella* associated with MSM transmission in Barcelona, 2020–21: outbreak of XRD *Shigella sonnei* and dissemination of ESBL-producing *Shigella flexneri*

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Background: Several countries have recently reported the detection of ESBL-producing *Shigella sonnei* associated with transmission among MSM. In a previous study by our group, 2.8% of *Shigella* spp. obtained from MSM in Barcelona between 2015 and 2019 were ESBL producers.

Objectives: To describe and characterize the emerging ESBL-producing *Shigella* spp. associated with sexual transmission among MSM detected from 2020 to 2021 in Barcelona, elucidating their connectivity with contemporaneous ESBL-producing *Shigella* spp. from other countries.

Results: From 2020 to 2021, we identified that among MSM, 68% of *S. sonnei* were XDR harbouring $bla_{\text{CTX-M-27}}$ and 14% of *Shigella flexneri* were MDR harbouring $bla_{\text{CTX-M-27}}$. WGS analysis showed that the ESBL-producing *S. sonnei* were part of a monophyletic cluster, which included isolates responsible for the prolonged outbreak occurring in the UK. Our data also reveal the first emergence and clonal dissemination of ESBL-producing and fluoroquinolone-resistant *S. flexneri* 2a among MSM.

Conclusions: We report an increasing trend of antimicrobial resistance in *Shigella* spp. among MSM in Barcelona since 2021, mainly as a consequence of the dissemination of XDR ESBL-producing *S. sonnei*, previously reported in the UK. These results highlight the importance of international collaborative surveillance of MDR/XDR *S. sonnei* and *S. flexneri* for rapid identification of their emergence and the prevention of the transmission of these pathogens.

Introduction

In high-income countries, shigellosis is mainly found in travellers returning from high-risk regions and as a sexually transmitted infection (STI) in MSM. Outbreaks of *Shigella sonnei* and *Shigella flexneri* among MSM have been reported worldwide.[1–6] According to international guidelines, the antibiotic therapy recommended for shigellosis includes ciprofloxacin, azithromycin, trimethoprim/sulfamethoxazole (co-trimoxazole) or ceftriaxone as a first/second-line treatments.[7,8] During the last decade, an increase in the number of cases of shigellosis in MSM, especially by isolates resistant to oral treatments, has been documented. Recently, the emergence of ESBL-producing *S. sonnei* has been reported in several countries.[1,9–11] In a rapid risk assessment released in February 2022, the ECDC raised the level of risk of shigellosis due to MDR *S. sonnei* for MSM to moderate, based on the high probability of infection but the low impact of such infection.[10]

In a previous study conducted by our group, we showed that among MSM-associated *Shigella* spp. collected in Barcelona from 2015 to 2019 only 2.8% were ESBL producers as they carried $bla_{CTX\text{-}M\text{-}27}$.[12] The aim of this study was to describe and characterize the emerging ESBL-producing *Shigella* isolates associated with sexual transmission among MSM detected from 2020 to 2021 in Barcelona, elucidating their connectivity with contemporaneous ESBL-producing *Shigella* spp. from other countries.

Methods

Study population and bacterial isolates

From January 2020 to December 2021, 37 *S. sonnei* and 43 *S. flexneri* were obtained from stool cultures of patients with diarrhoeal disease who attended primary healthcare units in Barcelona and different departments of the Vall d'Hebron Hospital, including the Sexually Transmitted Infections Unit, the Tropical Medicine and International Health Unit, and other medical departments. Duplicate isolates from the same individual were excluded. Patients' medical records were reviewed to collect their sociodemographic characteristics and to identify if pathogen acquisition was related to a sexually transmitted infection among MSM or to other sources.

Antibiotic susceptibility testing (AST)

AST using selected antimicrobials was performed by disc diffusion or the gradient diffusion method (Etest™, bioMérieux). MICs were interpreted according to the EUCAST clinical breakpoints, except for azithromycin for which epidemiological cut-off values were used (https://eucast.org/). MDR isolates were defined as those with resistance to ≥3 antibiotic categories and XDR as those with susceptibility to only one or two categories of antibiotics.[13]

WGS and bioinformatic analysis

WGS was performed in all MSM-associated *S. flexneri* and *S. sonnei* as well as all ESBL-producing *Shigella* obtained, regardless of the route of acquisition. Short-read sequencing for each isolate was performed by Nextera DNA Flex and MiSeq (Illumina) according to the manufacturer's instructions. Trimmomatic (v0.38), Unicycler (v0.4.7) and SPAdes (v3.14.1) were used to perform raw-read trimming and *de novo* genome assembly. Shigatyper (v1.0.5), Mykrobe (v0.9.0) and Resistance Gene Identifier (RGI, v4.2.2) were used for *in silico S. flexneri* serotyping, *S. sonnei* genotyping and for the identification of acquired antimicrobial resistance genes and point mutations associated with antimicrobial resistance, respectively.

The presence of the plasmid p183660 (accession no. KX008967), which has previously been identified as simultaneously encoding antimicrobial resistance determinants to third-generation cephalosporins (3GCs) ($bla_{CTX\text{-}M\text{-}27}$), macrolides [*mph*(A) and *erm*(B)], sulphonamides (*sul1*), trimethoprim (*dfrA17*) and aminoglycosides (*aadA5*) in *Shigella* was investigated by mapping the trimmed reads to the plasmid as reference with Snippy (v4.3.6).[14,15]

Based on the results from the short-read sequencing, representative MSM *Shigella* isolates encoding $bla_{CTX\text{-}M\text{-}27}$ and showing different resistance phenotypes were sequenced to characterize $bla_{CTX\text{-}M\text{-}27}$-harbouring plasmids by long-read sequencing using MinION technology (Oxford Nanopore Technologies). After DNA extraction using DNeasy PowerSoil Pro Kit (QIAGEN, Hilden, Germany), libraries were prepared using Native Barcoding Kit 96 v14 and sequenced on R10.4.1 flow cell (Oxford Nanopore Technologies). Basecalling was carried out with Guppy (v6.3.8) in standard mode. After trimming with Filtlong (v0.2.1), a hybrid assembly with short reads was performed with Unicycler (v0.4.8) and SPAdes (v3.14.1). Plasmids encoding $bla_{CTX\text{-}M\text{-}27}$ were compared with the plasmids pKSR100 (accession no. LN624486), p183660 and p893916 (accession no. MW396858), which have previously been identified to encode antimicrobial resistance determinants in MSM-associated *Shigella* isolates using BLAST Ring Image Generator (BRIG) and Mauve.[14,15]

Maximum-likelihood phylogeny was carried out with Snippy (v4.3.6) with *S. flexneri* 2a strain 2457T (AE014073) and *S. sonnei* 53G (NC_016822) as the reference strains, removing recombination events with Gubbins (v2.3.4). Finally, the single-nucleotide polymorphism (SNP)-based phylogenetic tree was constructed after 1000 bootstrap replicates with the IQ-Tree (v1.6.10) and tree annotation with relevant metadata was performed with the interactive tree of life (iTOL) (http://itol.embl.de). The genome of representative *Shigella* spp. isolates previously studied by our group and obtained from MSM from Barcelona in the period 2015–19 as well as the genome of international isolates obtained in previous studies were included to add context (Table S1, available as Supplementary data at *JAC* Online).

Data analysis

Categorical variables were compared using the chi-squared test (χ^2 test) or Fisher's exact test when appropriate.

Sequence data deposition

Reads used for this study were deposited in the National Center for Biotechnology Information (NCBI) under the BioProject number PRJNA864648.

Ethics

The study was approved by the Ethics Committee of the Vall d'Hebron Hospital, reference number PR(AG)456/2020. The study was conducted in accordance with the principles laid out in the Declaration of Helsinki and in accordance with the principles of Good Clinical Practice.

Results

Bacterial isolates and antimicrobial resistance

From 2020 to 2021, 37 *S. sonnei* and 43 *S. flexneri* were obtained from 80 patients in the Clinical Microbiology Department of the Vall d'Hebron Hospital. Of these, 59.5% of *S. sonnei* (22/37) and 65.1% of *S. flexneri* (28/43) were considered to have been acquired through sexual contact among MSM ($n=50$, median age 36.2 years, range 20–63) with no history of recent travel to low- or middle-income countries.

AST showed that 29% of *Shigella* spp. were ESBL producers. By species, 49% of *S. sonnei* (18/37) and 12% of *S. flexneri* (5/43) produced this resistance mechanism. By years, 33% of *S. sonnei* (5/15) were ESBL producers in 2020, and 59% in 2021 (13/22). Regarding *S. flexneri*, no ESBL producers were identified in 2020; however, 23% of the isolates obtained in 2021 (5/22) possessed this resistance mechanism. According to the source of acquisition, 83% (19/23) of ESBL-producing *Shigella* could be identified as acquired among MSM. The susceptibility to the remaining first-/second-line antimicrobials is shown in Table 1. MDR was found in 95% and 14% of MSM-associated *S. sonnei* and *S. flexneri*, respectively, while XDR was only detected among *S. sonnei* as 68% were co-resistant to azithromycin, ciprofloxacin, co-trimoxazole and cephalosporins (Table 1).

Antimicrobial resistance gene content analysis revealed that among the ESBL-producing *Shigella* isolates, 87% (20/23)

Table 1. Antimicrobial resistance rates of *S. sonnei* and *S. flexneri* isolates obtained in this study from 2020 to 2021, classified according to the source of acquisition

	S. sonnei			*S. flexneri*		
	MSM (*N* = 22) *n* (%)	Non-MSM/Unk (*N* = 15) *n* (%)	Total (*N* = 37) *n* (%)	MSM (*N* = 28) *n* (%)	Non-MSM/Unk (*N* = 15) *n* (%)	Total (*N* = 43) *n* (%)
Antimicrobial agent						
Ceftriaxone	15 (68)	3 (20)	18 (49)	4 (14)	1 (7)	5 (12)
Cefixime	15 (68)	3 (20)	18 (49)	4 (14)	1 (7)	5 (12)
Ertapenem	0	0	0	0	0	0
Ciprofloxacin	**21 (95)**	4 (27)	25 (68)	11 (39)	5 (33)	16 (37)
Co-trimoxazole	**22 (100)**	9 (60)	31 (84)	10 (36)	6 (40)	16 (37)
Azithromycin	**21(95)**	1 (7)	22 (59)	17(61)	5 (33)	22 (51)
MIC 16–48 mg/L	0	2 (13)	2 (5)	3 (11)	1 (7)	4 (9)
MIC ≥ 64 mg/L	21 (95)	1 (7)	22 (59)	14 (50)	4 (27)	18 (42)
MDR	**21 (95)**	3 (20)	24 (65)	5 (18)	1 (6)	6 (14)
XDR	**15 (68)**	1 (7)	16 (43)	0	0	0

Unk, unknown. *P* values <0.05 were considered statistically significant at the 95% CI level.
Significance found between acquisition groups within the same species, indicated by bold type.

encoded $bla_{CTX\text{-}M\text{-}27}$ and 13% (3/23) $bla_{CTX\text{-}M\text{-}15}$. All (19/19) MSM-ESBL-producing isolates encoded $bla_{CTX\text{-}M\text{-}27}$, while among the non-MSM/unknown origin isolates, 25% (1/4) harboured $bla_{CTX\text{-}M\text{-}27}$ and 75% (3/4) $bla_{CTX\text{-}M\text{-}15}$. Interestingly, three MSM-associated *S. sonnei* encoded $bla_{CTX\text{-}M\text{-}27}$ despite being susceptible to all the β-lactams tested and not showing evidence of ESBL production (i.e. synergy between amoxicillin/clavulanate and 3GCs). The genomic analysis of these isolates showed a disruption of the −35 (TTGAAA) and −10 (TACAAT) promoter regions of $bla_{CTX\text{-}M\text{-}27}$ due to the inversion of an IS*26* element located upstream (Figure 1, pSH280). Azithromycin-resistant isolates encoded *mph*(A) and/or *erm*(B), the co-trimoxazole resistant showed different combinations of the *dfrA1*, *dfrA17*, *sul1* and *sul2* genes and the ciprofloxacin-resistant isolates presented triple mutations in the QRDRs of the *gyrA* (S83L and D87G/N) and *parC* (S80I) genes. Other single mutations in QRDRs as well as plasmid-mediated quinolone resistance mediated by the Qnr family were also detected (Table S1).

Genomic analysis of $bla_{CTX\text{-}M\text{-}27}$-encoding plasmids

Plasmid alignment of the trimmed short reads revealed that all MSM-associated *S. sonnei* encoding $bla_{CTX\text{-}M\text{-}27}$ showed 95% coverage of p183660, while the MSM-associated *S. flexneri* encoding $bla_{CTX\text{-}M\text{-}27}$ exhibited a coverage of between 79% and 89%. Consequently, two MSM-associated *S. sonnei* (SH212 and SH280) and two *S. flexneri* (SH242 and SH282) encoding $bla_{CTX\text{-}M\text{-}27}$ and showing different resistance phenotype were selected for long-read sequencing to perform a detailed genomic analysis of $bla_{CTX\text{-}M\text{-}27}$-harbouring plasmids and to compare them with previously identified *Shigella* MSM-associated plasmids pKSR100, p183660 and p893916.

The $bla_{CTX\text{-}M\text{-}27}$-encoding plasmid from the *S. sonnei* isolates (pSH212 and pSH280) showed a high identity and coverage with plasmid pKSR100 (identity 98.11%; coverage 90%) described in a non-ESBL producer *S. flexneri* 3a from the UK, with p183660 (identity >99.97%; coverage 92%) previously identified from a CTX-M-27-producing *S. sonnei* isolate in the UK in 2015 and with plasmid p893916 (identity >99.98%; coverage 100%) described in a CTX-M-27-producing CipR.MSM5 genotype *S. sonnei* from the UK in 2020. On the other hand, the $bla_{CTX\text{-}M\text{-}27}$-encoding plasmid from the *S. flexneri* isolates (pSH242 and pSH282) also showed high identity with pKSR100, p183660 and p893916, but lower coverage than that found within the *S. sonnei* isolates (identity >97.9%; coverage 83%–90%; identity >99.9%; coverage 76%–86% and identity >99.89%; coverage 82%–93%, respectively).[14,15] The p183660 backbone was conserved in all plasmids, showing structural differences mainly due to deletions and genomic reorganizations of mobile genetic elements containing different combinations of antimicrobial resistance determinants. Specifically, pSH242 and pSHS282 from *S. flexneri* did not harbour the pKSR100 integron containing sulphonamide, trimethoprim and aminoglycoside resistance genes (*sul1*, *dfrA17* and *aadA5*), and additionally pSHS282 did not carry the genomic region containing the macrolide resistance determinants *mph*(A) and *erm*(B). Interestingly, $bla_{CTX\text{-}M\text{-}27}$ in *S. sonnei* was flanked by IS*26* and IS*15* while in *S. flexneri* it was flanked by two IS*26* (Figure 1 and Figure S1).

Genotyping, serotyping and genomic epidemiology of Shigella spp.

Genotyping of XDR ESBL-producing *S. sonnei* isolates according to the Hawkey's classification showed that 100% (15/15) of MSM-associated isolates belonged to the CipR.MSM5 genotype (genotype number 3.6.1.1.2) [Figure 2(a)].[16] The three non-MSM/unknown origin ESBL-producing *S. sonnei* belonged to genotypes CipR.MSM5, MSM4 (genotype number 3.7.25) and Central Asia III (genotype number 3.6), respectively [Figure 2(a)]. It was of note that the non-MSM-associated *S. sonnei* belonging to genotype

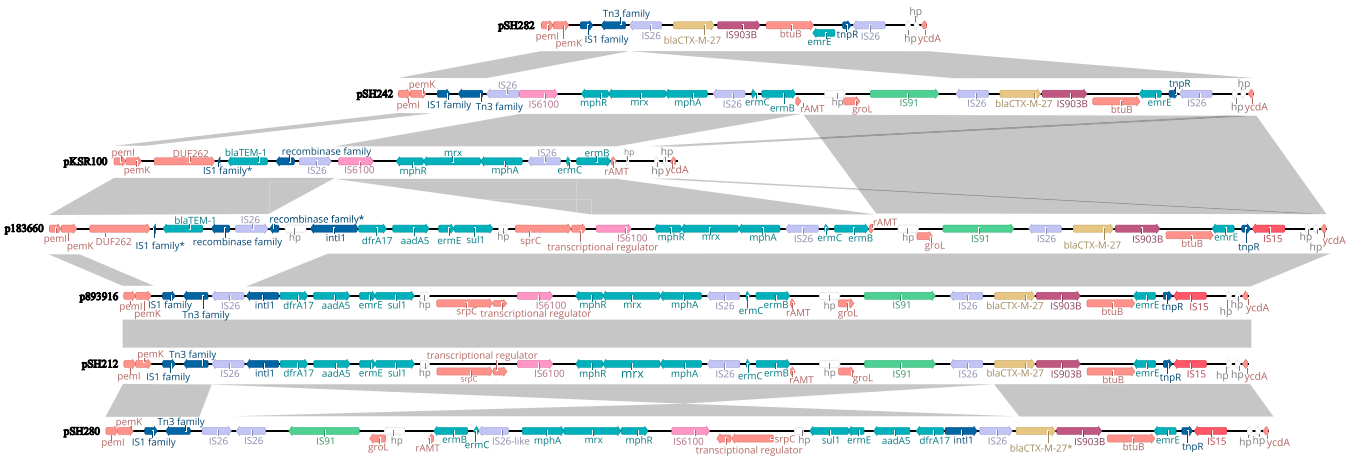

Figure 1. Structural organization of the region encoding antibiotic resistance determinants of $bla_{CTX\text{-}M\text{-}27}$-harbouring plasmids of *S. sonnei* and *S. flexneri* from this study compared with MSM-associated *Shigella* plasmids pKSR100, p183660 and p893916. pSH282 and pSH242 are plasmids of *S. flexneri* SH282 and SH242, respectively; pSH212 and pSH280 are plasmids of *S. sonnei* SH212 and SH280, respectively. Light grey shading denotes shared regions of identity. DUF denotes domain-containing proteins. * denotes partial features. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

CipR.MSM5 was obtained from a female in the context of an intrafamilial transmission event involving an MSM positive for *S. sonnei* (SNP distance = 1) (Figure 2). Regarding the MSM-associated non-ESBL-producing *S. sonnei* isolates, 86% (6/7) belonged to the genotype CipR.MSM5 and 14% (1/7) to the CipR.parent (genotype number 3.6.1).

The phylogeny of the *S. sonnei* isolates belonging to the 3.6 clade showed that all the XDR ESBL-producing CipR.MSM5 isolates obtained in this study nested together and were genetically closer to the representative XDR CipR.MSM5 isolates obtained in the UK (SRR11206407) and Australia (SRR12132003) (range 6–11 SNPs) than to the non-ESBL-producing CipR.MSM5 *S. sonnei* isolates obtained in Barcelona between 2015 and 2019 (range 12–42 SNPs) [Figure 2(b)].[1,15] On the contrary, the non-ESBL-encoding CipR.MSM5 isolates obtained between 2020 and 2021 were genetically closer to the CipR.MSM5 isolates obtained in Barcelona in the period 2015–19. Finally, the three isolates encoding $bla_{CTX\text{-}M\text{-}27}$ but not showing an ESBL phenotype also belonged to CipR.MSM5 and were clustered together (range 1–3 SNPs) [Figure 2(b)].

The *in silico* serotyping of MSM-associated *S. flexneri* showed that 46% (13/28) belonged to the 2a serotype, 27% (8/28) to 3a, 21% (6/28) to 1b and 4% (1/28) to 1c. All the ESBL-producing *S. flexneri* isolates (5/5) belonged to the 2a serotype. The four MSM-associated ESBL-producing *S. flexneri* isolates formed a closely related independent cluster within the phylogeny (range 0–15 SNPs) and no epidemiological link was identified among the patients from whom these isolates were obtained. The remaining non-MSM-associated ESBL-producing isolates showed a higher genetic distance from them (range 200–204 SNPs) (Figure 3). The four MSM-associated ESBL-producing isolates possessing a triple mutation in the QRDR [*gyrA* (S83L and D87N) and *parC* (S80I)] were genetically close to the non-ESBL-producing fluoroquinolone-resistant *S. flexneri* identified in Barcelona in the 2015–19 and 2020–21 periods, in Australia in 2016 (SRR7885970) and in the UK in 2020 (SRR12175226) (Figure 3).[17,18]

Discussion

We describe an increase and dissemination of XDR ESBL-producing *S. sonnei* and MDR ESBL-producing *S. flexneri* among MSM in Barcelona during 2020–21. Specifically, 68% of *S. sonnei* and 14% of *S. flexneri* acquired by MSM were ESBL producers and simultaneously resistant to ciprofloxacin, azithromycin and/or co-trimoxazole. These findings differ from the data previously obtained by our group in 2015–19, in which only 3.8% of *S. sonnei* and 2.3% of *S. flexneri* acquired among MSM were ESBL producers.[12]

ESBL-producing *S. sonnei* among MSM has been detected in several countries. Australia reported a prolonged outbreak of ESBL-producing *S. sonnei* belonging to the VN2.KH1.Aus genotype (genotype number 3.7.29.1.4.1) from 2019 to 2020, mainly associated with males.[1] In addition, the MSM-related ESBL-producing isolates detected in Canada and in Belgium between 2017–19 and 2018–19, respectively, and the unique ESBL-producing *S. sonnei* isolate detected in Barcelona during 2015–19 also belonged to this genotype.[4,12,19] Nevertheless, sporadic detection of XDR ESBL-producing CipR.MSM5 isolates were also identified in Australia and in the UK before the COVID-19 epidemic.[1,11] However, an outbreak of XDR ESBL-producing CipR.MSM5 *S. sonnei* was detected in the UK between September 2021 and February 2022.[11] In our previous study, we identified that the most prevalent MSM-related *S. sonnei* genotype detected during 2015–19 in Barcelona was CipR.MSM5.[12] Despite not being ESBL-producers, isolates belonging to this genotype have shown great expansion in recent years, as they have also been found as being the most prevalent among the ciprofloxacin- and azithromycin-resistant isolates in Australia, the UK and the USA during 2018–19 and also the most prevalent in MSM-associated shigellosis in Belgium.[4,16] This may demonstrate successful worldwide dissemination of this genotype, even before ESBL acquisition.

In our present study, three MSM-associated XDR ESBL-producing CipR.MSM5 isolates were sporadically detected

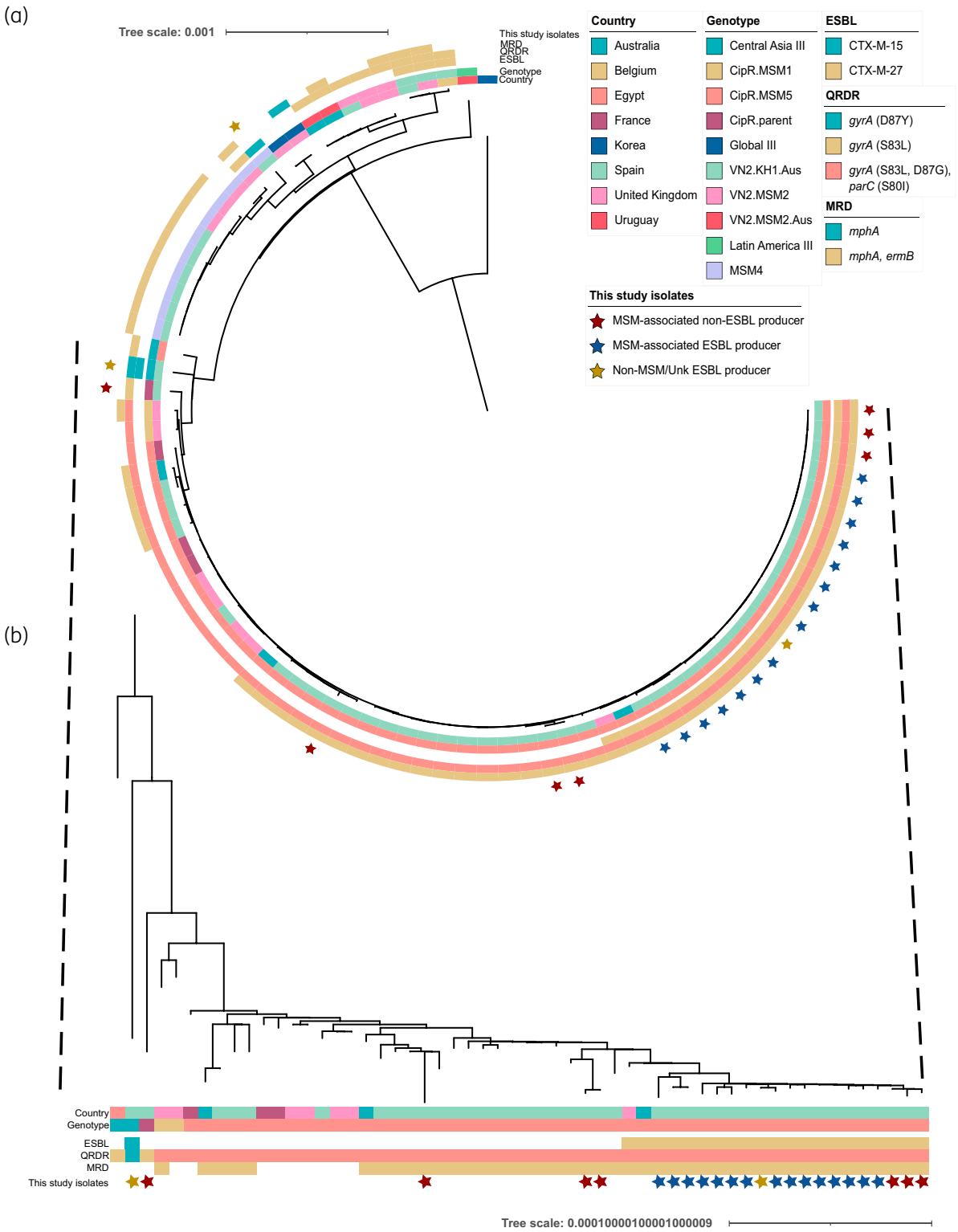

Figure 2. Phylogenomic analysis of *S. sonnei* isolates. (a) Maximum-likelihood tree of the ESBL-producing *S. sonnei* and MSM-associated *S. sonnei* isolates collected in Barcelona in this study from 2020 to 2021 ($n=25$), as well as the MSM-associated *S. sonnei* isolates previously identified in the 2015–19 period in Barcelona ($n=26$) and the selected international representative *S. sonnei* isolates ($n=27$). The tree was rooted with *S. sonnei* 53G. (b) Phylogeny of the *S. sonnei* isolates belonging to the 3.6 clade based on the Hawkey *et al.* classification. Isolate metadata and molecular characteristics are colour coded, as detailed in the key. MRD, macrolide resistance determinant. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

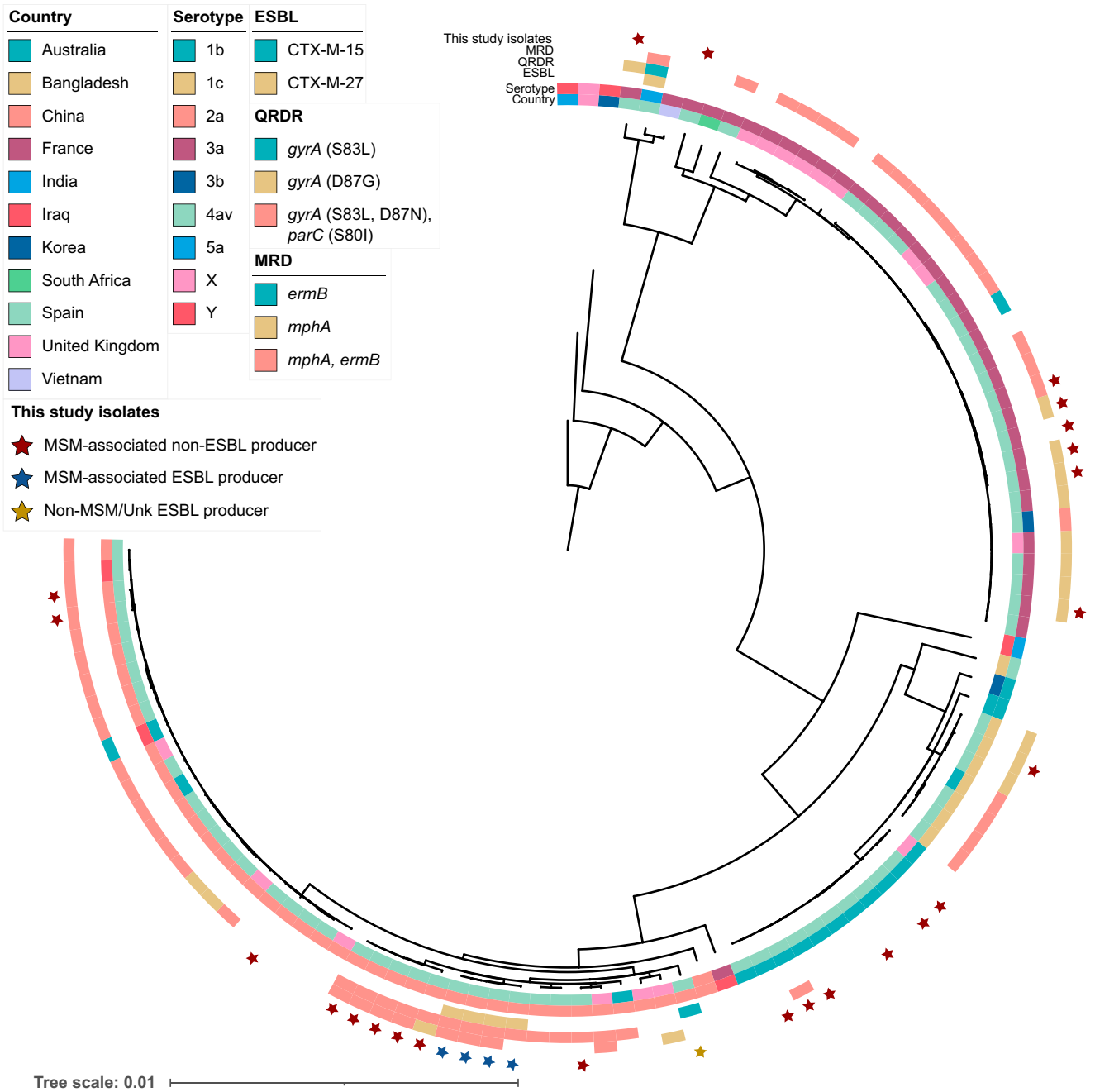

Figure 3. Phylogenomic analysis of *S. flexneri* isolates. Maximum-likelihood tree of the ESBL-producing *S. flexneri* and MSM-associated *S. flexneri* isolates collected in Barcelona in this study from 2020 to 2021 ($n=29$), as well as the MSM-associated *S. flexneri* isolates previously identified in the 2015–19 period in Barcelona ($n=44$) and the selected international representative *S. flexneri* isolates ($n=31$). The tree was rooted with *S. flexneri* 2a strain 2457T. Isolate metadata and molecular characteristics are colour coded, as detailed in the key. MRD: macrolide resistance determinant. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

in December 2020 in Barcelona; however, in June 2021 a re-appearance and rapid outbreak expansion was detected. The *S. sonnei* phylogeny revealed that all the outbreak-related XDR CipR.MSM5 isolates from Barcelona were part of the same monophyletic cluster in which the representative XDR CipR.MSM5 isolates from the UK and Australia were also found. This fact reinforces the international transmission hypothesis by which this clone has been introduced in our country, replacing the non-ESBL-producing CipR.MSM5 clone, which previously circulated in our area, and originating a local outbreak in Barcelona. The successful dissemination of this clone among different countries may be the consequence of the interconnected international sexual networks among MSM after the relaxation on the COVID-19 epidemic measures in the summer of 2020.[11,12,20]

On the other hand, the genomic analysis of $bla_{CTX\text{-}M\text{-}27}$-encoding plasmids in this study showed a high similarity of these mobile genetic elements with previous plasmids encoding resistance to macrolides, co-trimoxazole, aminoglycosides and/or β-lactams in MSM-associated *Shigella* (pKSR100, p183660 and p893916). The pKSR100 plasmid, which among others encodes macrolide resistant determinants, was proposed to have had a role in driving the epidemics of azithromycin-resistant *S. sonnei* and *S. flexneri* among MSM.[21] Thus, the combination of pKSR100-type plasmids that have acquired $bla_{CTX\text{-}M\text{-}27}$ with a successfully disseminated genotype of *S. sonnei* could have facilitated the expansion and increase of XDR ESBL-producing *Shigella* among MSM during the last 2 years. Additionally, the location of several resistance genes within the same plasmid could also be an element that may contribute to its co-selection and dissemination, especially in contexts of high antibiotic pressure such as MSM receiving treatment for other STIs such as *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infection, as has previously been documented in the UK.[11]

Unlike *S. sonnei*, no MSM-associated outbreaks of ESBL-producing *S. flexneri* have previously been reported. In our study, we identified four MSM-associated CTX-M-27-producing *S. flexneri* 2a isolates. As no epidemiological link was detected among the patients from whom these isolates were obtained and they showed a close genetic distance, the existence of secondary non-detected cases could be considered. $bla_{CTX\text{-}M\text{-}27}$ in *S. flexneri* isolates was found in a pKSR100-related plasmid and showed a high identity with plasmids harbouring $bla_{CTX\text{-}M\text{-}27}$ in *S. sonnei*. Consequently, the hypothesis of the acquisition by *S. flexneri* of pKSR100-type encoding the $bla_{CTX\text{-}M\text{-}27}$ plasmid circulating among *S. sonnei* would be plausible. For this to occur, several genomic reorganizations involving the loss of several antimicrobial resistance genes should have happened. Another possible scenario could have been the acquisition of a genomic fragment containing $bla_{CTX\text{-}M\text{-}27}$ by a circulating pKSR100-type plasmid in *S. flexneri* mediated by the IS*26* elements flanking this structure. These hypotheses have yet to be confirmed. In any case, it is necessary to continue monitoring the evolution of ESBL production in *S. flexneri* isolates in MSM in our environment, as well as their possible emergence or dissemination in other countries.

In this study we identified a significant increase of quinolone resistance in *S. flexneri* in MSM compared with what we observed in the 2015–19 period (4.5% versus 38%) as a consequence of the introduction of fluoroquinolone-resistant *S. flexneri* 2a ($P < 0.05$).[12] Although the MSM-associated ESBL-producing *S. flexneri* detected in Barcelona should not be considered XDR, as simultaneous resistance to co-trimoxazole and azithromycin has not yet been found, these results should warn of the possibility that *S. flexneri* could also acquire additional resistance determinants, thereby following the same antibiotic resistance evolution as the MSM-related *S. sonnei*. This could be a plausible phenomenon that might happen by horizontal gene acquisition of such resistance determinants in a population with high antimicrobial exposure.

One limitation of this study is that due to the retrospective design of the research, some demographic and epidemiological data are missing. Specifically, data regarding sexual contacts or information about travel to non-low- or -middle-income countries by the cases included was not collected, and consequently, it was not possible to establish epidemiological links between the patients and/or identify possible transmission chains.

In summary, in this work we report an increasing trend of antimicrobial resistance in *Shigella* spp. among MSM in Barcelona since 2021, mainly as a consequence of the emergence of an outbreak of XDR ESBL-producing *S. sonnei* belonging to the CipR.MSM5 genotype. Our data demonstrate a close genetic link with the XDR ESBL-producing *S. sonnei* isolates responsible for the prolonged outbreak detected in the UK also in 2021, confirming its successful international dissemination coinciding with the increase in social interactions after the COVID-19 lockdown. Additionally, to our knowledge, we have detected the first clonal dissemination of MDR ESBL-producing *S. flexneri* among MSM, which also represents a significant health threat. These results reinforce the importance of collaborative international surveillance of MDR/XDR *Shigella* spp. for rapid identification of its emergence as well as the need to implement measures that allow early diagnosis and contact tracing of cases of shigellosis among MSM to reduce its dissemination.

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Transparency declarations

All authors declare that they have no conflicts of interest to disclose.

Supplementary data

Figure S1 and Table S1 are available as Supplementary data at *JAC* Online.

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