High frequency of increased triclosan MIC among CC5 MRSA and risk of misclassification of the SCCmec into types

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Background: Typing of staphylococcal cassette chromosome mec (SCCmec) elements is commonly used for studies on the molecular epidemiology of MRSA.

Objectives: To perform an investigation centred on uncovering the reasons for misclassification of MRSA clonal complex 5 (CC5) SCCmec type II clinical isolates in our laboratory.

Methods: MRSA isolates from CC5 were subjected to WGS and SCCmec typing.

Results: This investigation led to the discovery that the classification failure was due to an insertion of IS1272 carrying the fabI gene on a transposable element (TnSha1) that confers increased MIC to the biocide triclosan. Genomic analysis revealed that fabI was present in 25% of the CC5 MRSA isolates sampled. The frequency of TnSha1 in our collection was much higher than that observed among publicly available genomes (0.8%; n=24/3142 CC5 genomes). Phylogenetic analyses revealed that genomes in different CC5 clades carry TnSha1 inserted in different integration sites, suggesting that this transposon has entered CC5 MRSA genomes on multiple occasions. In at least two genotypes, ST5-SCCmecII-1539 and ST5-SCCmecII-12666, TnSha1 seems to have entered prior to their divergence.

Conclusions: Our work highlights an important misclassification problem of SCCmecII in isolates harbouring TnSha1 when Boye’s method is used for typing, which could have important implications for molecular epidemiology of MRSA. The importance of increased-MIC phenotype is still a matter of controversy that deserves more study given the widespread use of triclosan in many countries. Our results suggest expanding prevalence that may indicate strong selection for this phenotype.

Introduction

In addition to the meticillin resistance encoded by mec gene alleles present in staphylococcal cassette chromosome mec (SCCmec), MRSA frequently display resistance to other antimicrobials and increased MICs of biocides.¹⁻³ For biocides, the commonly used concentrations are higher than the MICs tested in laboratory. Thus, biocide failures would seem improbable. However, many biocides have at least two distinct mechanisms of action. In low concentrations they interact with specific targets inhibiting bacterial growth, whereas at high concentrations they promote membrane disruptions or protein denaturation.⁴ The increased-MIC phenotype is most commonly related to target gene modifications.

Triclosan is a synthetic chlorinated phenolic agent commonly used in commercial and healthcare products including soaps, shampoos, deodorants, toothpaste, cosmetics, hospital disinfectants, fabrics, surgical sutures and prosthetics⁵ and is regularly used in hospital environments for MRSA decontamination and for controlling outbreaks.⁶ In the USA, this biocide was banned in domestic antiseptic washes.⁶ However, in Brazil there is no restriction for the use of this biocide and the most recent regulation of its use was established in 2012.⁷ Triclosan targets the biosynthesis of fatty acids by irreversible inhibiting enoyl-acyl carrier protein reductase, the product of the fabI gene, by mimicking its natural substrate.⁸ One of the mechanisms of increased triclosan MIC is the acquisition of a heterologous copy of the fabI gene.
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The expression of which leads to increased amounts of the triclosan target molecule, reducing the bacterial susceptibility to this biocide.11 Another important mechanism is the acquisition of mutations in the intrinsic copy of fabI.12

In *S. aureus*, the heterologous fabI gene (named sh-fabI) is carried by the transposon TnSha1, which is mobilized by a copy of IS1272, a member of the IS1182 family of ISs.13

Despite the common use of triclosan at high doses,8 there are some concerns that the exposures to residual concentrations may select strains with increased MIC since concentrations may vary depending on the type of surfaces treated over different periods of time. There are also a few reports of high-dose resistance to this biocide, which may lead to failure in disinfection processes.14 Another important concern is the possibility that strains with increased MIC may be more prone to becoming resistant to higher doses.15–17

Increased MIC of triclosan is mediated by TnSha1, which possibly originated from *Staphylococcus haemolyticus*.11 Until recently, the frequency of this phenotype in *Staphylococcus aureus* was very low.12 Here we report the emergence of increased-MIC phenotype to triclosan in MRSA isolates from the CC5 lineage, isolate CR15-071 (Acc. CP065868), carrying TnSha1 with the location of its IS1272 (outside SCCmec element) at position 2079331–2081013 (blue arrow). Blue arrows represent IS1272, which is present in SCCmecIV but absent in SCCmecII, and the ccrAB gene, which is present in both mec cassettes. Blue gradient indicates nucleotide identity percentage between sequences. The figure was created using BRIG 0.95 and Easyfig 2.1 software. This figure appears in colour in the online version of *JAC*.

**Materials and methods**

**Bacterial isolates**

One hundred and eighty MRSA isolates from CC5 were included in this study. These isolates belong to a collection of 600 MRSA obtained from 51 hospitals located in the Rio de Janeiro city metropolitan area.18 Clinical data and other information about this collection is available in Table S1 (available as Supplementary data at *JAC* Online). A complete and comprehensive version of the Materials and methods is presented in File S1.

**MRSA molecular typing**

Initially, the CC5 isolates were typed using a PCR-based restriction modification (RM) test.19,20 SCCmec typing was performed using oligonucleotides and multiplex PCR (mPCR) conditions developed by Boye et al.21 In addition, all isolates typed as SCCmecIV by Boye’s method were tested by PCR using the primers that target the kdp gene, previously described as a marker for the SCCmecII (Table S2).22

**Genomic analyses**

WGS was previously performed by our group using Illumina HiSeq (Illumina, San Diego, CA, USA).18 Reads were trimmed using BBduk Trimmer (v1.0) and genome assembly performed using Velvet Assembly (v7.0.4).23 Molecular characterization was performed on MLST 2.0, SCCmecFinder and spaTyper 1.0 (Center for Genomic
Epidemiology; http://cge.cbs.dtu.dk). The genome sequence of the isolate CR15-071 was completely assembled using Geneious Prime software. A BLAST command line application was used to search for IS1272 and the sequence of sh-fabI. TnSha1 was also searched in a local database (https://data.mendeley.com/datasets/mkwvsp8rhg/1) comprising 8324 genomes of S. aureus related to the most frequent MRSA lineages using BLAST command line. Additional searches for TnSha1 were performed on the NCBI database using BLAST. All genomes found to carry a TnSha1 sequence in our 8324 genome database were also queried for resistance genes using the ResFinder 3.1 data-base.

Results

Molecular typing

Among the CCS isolates typed by Boye’s method, 54.4% (n=98) were classified as CC5-SCCmecIV based on the amplification of the segment of IS1272. The remaining 45.6% (n=82) were typed as CC5-SCCmecII based on the amplification of a segment corresponding to ccrA2-B and the absence of products for IS1272. To our surprise, SCCmec typing of the genome sequences using SCCmecFinder displayed contradictory results. A total of 32 isolates previously typed as SCCmecII by Boye’s method were typed as SCCmecI by SCCmecFinder (Table S1). According to the genome-based SCCmec typing, the correct percentage of SCCmecII in this collection was 63.3% (n=114/180) and SCCmecIV accounted for 36.7% (n=66/180). All of these 32 SCCmecII isolates, misclassified as SCCmecIV by Boye’s method, amplified the expected kdp product, which is only present in isolates carrying SCCmecII.

Genomic analysis

Examining the genomes of previously misclassified SCCmecII isolates, a new copy of IS1272 was found outside SCCmec in all of
apparently did not hinder primer annealing by the primers developed to detect SCCmec showed that all of them carry TnSha1. Of all these IS elements, 98.0% coverage and 99.6% nucleotide identity with mecSCCmecIV, showed that all of them carry Tn1272. All of these IS

| Clone               | TnSha1+ | Total |
|---------------------|---------|-------|
| ST5-SCCmecII-t539   | 17      | 17    |
| ST1635-SCCmecIV-t002| 10      | 10    |
| ST105-SCCmecII-t002 | 7       | 73    |
| ST5-SCCmecII-t2666  | 5       | 5     |
| ST1635-SCCmecIV-NT  | 1       | 1     |
| ST1635-SCCmecIV-t450| 1       | 1     |
| ST1635-SCCmecIV-t062| 1       | 1     |
| ST1635-SCCmecIV-t769| 1       | 1     |
| ST5-SCCmecII-NT     | 1       | 1     |
| ST4876-SCCmecII-t002| 1       | 2     |
| ST105-SCCmecII-t005 | 1       | 5     |
| ST5-SCCmecIV-t002   | 2       | 43    |
| ST5-SCCmecII-t067   | 0       | 6     |
| ST5-SCCmecIV-NT     | 0       | 6     |
| ST105-SCCmecII-t100 | 0       | 2     |
| ST1635-SCCmecIV-t1154| 0      | 2     |
| ST105-SCCmecII-t067 | 0       | 1     |
| ST5-SCCmecII-t539   | 0       | 1     |
| ST5-SCCmecII-t002   | 0       | 1     |
| ST5-SCCmecIV-t061   | 0       | 1     |
| ST5-SCCmecIV-t062   | 0       | 1     |
| ST5-SCCmecIV-t105   | 0       | 1     |
| ST5-SCCmecIV-t586   | 0       | 1     |
| ST5-SCCmecIV-t777   | 0       | 1     |
| Total               | 48      | 180   |

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|---------------------|---------|-------|
| ST5-SCCmecII-t539   | 17      | 17    |
| ST1635-SCCmecIV-t002| 10      | 10    |
| ST105-SCCmecII-t002 | 7       | 73    |
| ST5-SCCmecII-t2666  | 5       | 5     |
| ST1635-SCCmecIV-NT  | 1       | 1     |
| ST1635-SCCmecIV-t450| 1       | 1     |
| ST1635-SCCmecIV-t062| 1       | 1     |
| ST1635-SCCmecIV-t769| 1       | 1     |
| ST5-SCCmecII-NT     | 1       | 1     |
| ST4876-SCCmecII-t002| 1       | 2     |
| ST105-SCCmecII-t005 | 1       | 5     |
| ST5-SCCmecIV-t002   | 2       | 43    |
| ST5-SCCmecII-t067   | 0       | 6     |
| ST5-SCCmecIV-NT     | 0       | 6     |
| ST105-SCCmecII-t100 | 0       | 2     |
| ST1635-SCCmecIV-t1154| 0      | 2     |
| ST105-SCCmecII-t067 | 0       | 1     |
| ST5-SCCmecII-t539   | 0       | 1     |
| ST5-SCCmecII-t002   | 0       | 1     |
| ST5-SCCmecIV-t061   | 0       | 1     |
| ST5-SCCmecIV-t062   | 0       | 1     |
| ST5-SCCmecIV-t105   | 0       | 1     |
| ST5-SCCmecIV-t586   | 0       | 1     |
| ST5-SCCmecIV-t777   | 0       | 1     |
| Total               | 48      | 180   |

NT, non-typeable.

them. The genome of one of these isolates, CR15-071 (ST5-SCCmecII-t539; Acc: CP065868), was closed and annotated. This new IS1272 was located at position 20799331-2081013 outside the mec cassette, which is located at position 31479 to 30000 in the genome of CR15-071 (Figure 1).

Close examination of the new IS1272 in CR15-071 revealed the presence of the gene sh-fabi, the determinant of the increased-triclosan-MIC phenotype in staphylococci, located upstream and adjacent to IS1272, thus indicating an insertion of the TnSha1 transposon (Figure 2). The nucleotide alignment of sh-fabi in CR15-071 and the original fabI gene carried by S. haemolyticus (Acc. CP011116.1) showed only one synonymous (C231T) mutation (Figure 2).

Genomic searches for sh-fabi sequences in the 32 Brazilian misclassified isolates, because they carry a IS1272 not linked to SCCmec, showed that all of them carry TnSha1. All of these IS1272 elements had 98.0% coverage and 99.6% nucleotide identity with those inserted in the SCCmecIV locus. These sequence differences apparently did not hinder primer annealing by the primers developed by Boye et al. (Figure 2), explaining the SCCmecIV misclassification as SCCmecIV detected in this work. TnSha1 was distributed in similar proportions among isolates carrying SCCmecII (28.1%; n = 32/114) or SCCmecIV (24.2%; n = 16/66). For isolates carrying SCCmecIV, this transposon was distributed in the lineages ST5-SCCmecII-t539 (n = 17/17), ST5-SCCmecII-t2666 (n = 5/5), ST5-SCCmecII spa not typeable (NT) (n = 1/1), ST105-SCCmecII-t002 (n = 7/73), ST105-SCCmecII-tNT (n = 1/5) and ST4876-SCCmecII-t002 (n = 1/2).

Among SCCmecIV isolates, TnSha1 was found in the lineages ST1635-SCCmecIV-t002 (n = 10/10), while the remaining isolates (n = 4/4) belonged to the same ST but with miscellaneous spa types or were genotyped as ST5-SCCmecIV-t002 (2/43) (Table 1).

TnSha1-positive isolates were found in 22 of the 34 hospitals analysed (almost 70.0%), in both public (45.5%; n = 10) and private (54.5%; n = 12) hospitals. The distribution of this gene showed an increased prevalence in the genome of non-bloodstream infection (non-BSI) MRSA isolates (38.2%; n = 21/55) followed by the isolates collected from anterior nares (26.8%; n = 15/56), and were least prevalent in BSI (17.4%; n = 12/69) [Figure 3(a); P = 0.0340].

The percentage of resistance-associated genes in the TnSha1-negative genomes was lower for chloramphenicol (26.5%; n = 35/132), trimethoprim (1.5%; n = 2/132) and tetracycline (0.8%; 1/132); however, for aminoglycosides (n = 128/132; 97.0%) and macrolides (n = 121/132; 91.7%) the rates were higher (P < 0.0208) [Figure 3(b)].

Our analysis suggests that TnSha1 has entered into CC5 MRSA genomes on several different occasions (at least eight independent events of insertions) at five distinct locations on the CC5 genomes. A more basal subclade formed by ST1635-SCCmecIV (blue branches; n = 13) had TnSha1 inserted into the same location at 1310 bp upstream from a hypothetical protein CDS. However, another subclade formed by genomes ST5-SCCmecII (green branches; n = 22) had TnSha1 integrated between the ORFs coding for a hypothetical protein and a Class A β-lactamase. These data suggest at least two independent episodes of clonal spread of TnSha1. Indeed, TnSha1 was inserted into some other genomes of the tree through independent events (e.g. UB501 and CR15-039, ST5-SCCmecIV; and UB466, ST5-SCCmecIV; purple branches) (Figure 4).

Two distinct integration sites were identified amongst ST105-SCCmecII genomes carrying TnSha1 (n = 9). One site was located between a resolvase/integrase CDS and a specific hypothetical protein CDS (pink branches; n = 4). Indeed, a ST4876-SCCmecII (ST105-related) isolate CHU15-086 also had TnSha1 integrated into this same site (pink branch). Another ST105-SCCmecII integration site was located between a site-specific recombinase CDS and a β-alanyl-β-alanine carboxypeptidase/PBP4 CDS (orange branches; n = 4) (Figure 4). Genomic coordinates of the CDS that flank TnSha1 integration sites are presented in Table 53.

Phylogenetic analysis also revealed that the TnSha1-positive MRSA were distributed in three main phylogenetic groups. The ST1635-SCCmecIV and ST5-SCCmecIV genomes clustered in the
most basal clade, corresponding to the CC5-Basal grouping, as described by Challagundla et al.\textsuperscript{27} The ST5-SCCmecII genomes clustered in a more derived clade, consistent with the CC5-II A phylogenetic group described by Challagundla et al.,\textsuperscript{27} and the ST105-SCCmecII genomes clustered in a clade corresponding to the CC5-II B phylogenetic group previously described.\textsuperscript{27} The ST105-SCCmecII TnSha1 positives were distributed in two subclades, each of them characterized by a specific transposon
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integration site (Figure 4). The Bayesian chronology showed that at least for ST105-SCCmecII-t002 genomes (the predominant clone in BSI in Rio de Janeiro18) the acquisition of TnSha1 was estimated to occur after 2013 (2012–14), a few years after the expansion of these isolates in Rio de Janeiro, which was estimated to have occurred in 2009 (2007–10)18 (Table 2).

Searching for TnSha1 carrying the sh-fabI gene in the NCBI databases

This study revealed that the complete TnSha1 is present in only 59 genomes (0.7%) of a database composed of 8324 genomes. Among these, 34 (57.6%) were from MRSA and 25 (42.4%) from MSSA. The most frequent ST carrying TnSha1 was (CC5)ST5 (n=26/59; 44.1%; Figure S1). Of the 34 genomes, 44.1% (n=15) carried SCCmec type II and 41.2% (n=14) carried SCCmec type IV. One TnSha1-positive genome carried SCCmecIII and four were considered non-typable. Most of the complete TnSha1-positive genomes found were isolated in the USA (n=36; 61.0%). Three genomes from Brazil, isolated in São Paulo, were also found in this database: UB466, ST5(CC5)-SCCmecII-t954 (Acc. GCA_003095355.1); MSSA UB501, ST5(CC5)-t002 (Acc. GCA_003075675.1) and MSSA-VRSA HOU-1444VR, ST5(CC5)-t002 (Acc. GCA_001278745.1). In addition, sh-fabI was found in 35 genomes lacking an adjacent IS1272 transposase (partial TnSha1). Most of these genomes belonged to ST8 (40%). The remaining genomes were classified as: ST22 (22.9%); ST239 (17.1%); ST5 (8.6%); ST36 (2.9%); ST30 (2.9%); and ST398 (2.9%). The genome UC50 did not have a defined ST. Regarding geographical location, these genomes came from the USA (42.9%), the UK (25.7%), Thailand (11.4%), Colombia (5.7%), Dominica (2.9%) and Denmark (2.9%). The country of isolation was not reported for two genomes (5.7%) (Figure S1).

The distribution of TnSha1 among other Staphylococcus species was also assessed by BLAST searches on NCBI database, restricted to the Staphylococcus genus (taxid 1279). Only complete TnSha1 sequences with a minimum 90% coverage and 90% nucleotide identity were considered. Thirty-seven Staphylococcus organisms (excluding S. aureus) carried TnSha1. The majority were S. haemolyticus (n=15) and Staphylococcus epidermidis (n=10). In addition, a few representatives (one to three

Figure 4. ML phylogenetic tree of 179 MRSA CCS genomes from Rio de Janeiro, Brazil, 2014–17 and of 482 reference genomes selected from Challagundla et al. (2018).27 Scale bar corresponds to an estimated evolutionary distance of 0.01. TnSha1-positive genomes are marked with coloured branches and shadow. Each different colour represents a different integration site in MRSA genomes. This figure appears in colour in the online version of JAC and in black and white in the print version of JAC.
Table 2. Estimated dates for the most recent ancestor (MRCA) of TnSha1-positive isolates from ST105-SCCmecII-t002 lineage of MRSA

| Date of MRCA (95% credibility) | ST105-SCCmecII-t002 (TnSha1 interval) |
|-------------------------------|---------------------------------------|
| 2014 (2013–2015)              | CD16-113                              |
| 2014 (2013–2015)              | CD15-126                              |
| 2014 (2013–2015)              | CD15-137                              |
| 2014 (2013–2015)              | CD15-152                              |
| 2013 (2012–2014)              | CHU15-088                             |
| 2011 (2009–2012)              | CHU15-086                             |
| 2012 (2011–2013)              | CD15-123                              |
| 2012 (2011–2013)              | CD16-180                              |
| 2012 (2011–2013)              | CD15-138                              |

The dates were estimated by chronology based on a Bayesian tree constructed previously with the genomes of these isolates. By this chronology ST105-SCCmecII-t002 was predicted to have expanded in Rio de Janeiro by 2009 (2007–2010).16

Table 3. Identity of the target sequence

| Identity used in our study | Description |
|---------------------------|-------------|
| sha1                      | TnSha1      |
| mecII                     | SCCmecII    |
| fbb1                      | sh-fbb1     |
| spoT                      | spoT hydrolase |
| pppGpp                     | ppGpp      |
| UTI89                      | UTI89      |
| MG1655                     | MG1655      |
| FPR3757                    | FPR3757     |
| USA300                     | USA300      |
| USA35                      | USA35      |
| ATCC                       | ATCC       |

Discussion

The results presented here reveal two new observations about the molecular and clinical epidemiology of hospital-associated (HA)-MRSA: the unusually high frequency of increased triclosan MIC values amongst CC5 isolates in the Rio de Janeiro area, and the misclassification of SCCmec for isolates carrying sh-fbb1 and SCCmecII with Boye’s method.21 Despite the high frequency of TnSha1 among CC5 isolates from Rio de Janeiro (26.7%; n = 48/180), from the CC5 genomes in a publicly available database we detected 0.76% (n = 24) TnSha1 among 3142 genomes analysed. Furi et al. (2016)13 found 133 complete or partial TnSha1 elements from their BLAST searches of the NCBI database. However, in our analysis, this number was lower (S. aureus, n = 51; Staphylococcus spp., n = 37). This discrepancy may be explained by the stricter cut-off value of 90% nucleotide sequence identity used in our study.

Other studies have described variable rates of triclosan resistance. In a mupirocin-resistant MRSA population, 2% of the isolates were resistant to triclosan,28 while in a collection of 186 S. aureus clinical isolates randomly selected from a microbiology laboratory in the UK this percentage was 7.5%.29 Contrary to the hypothesis that biocide resistance may also increase resistance to other antimicrobials,4,30,31 in our study, the resistance rates amongst TnSha1-positive and -negative genomes were similar in the case of chloramphenicol, or even higher for TnSha1-negative genomes in the case of macrolides and aminoglycosides. Only for trimethoprim and tetracycline were the resistance rates higher for TnSha1-positive isolates.

We detected TnSha1 in various CCS lineages in Rio de Janeiro. ST5-SCCmecII-t539, which accounts for most of the SCCmecII-TnSha1-positive isolates (n = 17/32; 53.1%), was also detected in São Paulo, Brazil32 and in other countries including China and Germany.33,34 The second most frequent lineage among SCCmecII-TnSha1-positive genomes was ST105-SCCmecII-t002 (n = 7/32; 21.9%). In the last 15 years, ST105-SCCmecII has sporadically been reported in São Paulo,32 the USA35 and in Portugal.36 For SCCmecIV MRSA, TnSha1 was more frequently detected amongst ST1635-SCCmecIV-t002 (n = 10/16; 62.5%). This clonal lineage seems to have recently emerged in Brazil, and has also been reported in Vitória, Espírito Santo state,37 Rio de Janeiro, Rio de Janeiro state38 and Botucatu, São Paulo state.39 However, none of these reports searched for triclosan resistance. In a previous work18 with these same 180 CC5 isolates, ST105-SCCmecII isolates were mainly isolated from blood. Thus, the low incidence of TnSha1 among isolates from BSI was possibly due to the relatively low incidence of this element among ST105-SCCmecII isolates in the total TnSha1-positive genomes (14.6%; n = 7/48).

ST1635-SCCmecIV-t002 genomes are a part of the most basal clade in our phylogenetic tree. This lineage clustered with ST5-SCCmecIV-t002 genomes, in the CC5-Basal group defined by Challagundla et al.27 Nevertheless, the integration site of TnSha1 characterized in ST1635 genomes is different from the sites found in the other CC5 genotypes, including the genetically related ST5-SCCmecIV, indicating likely independent TnSha1 acquisitions. However, despite this evidence for convergent evolution, clonal (vertical) spread of TnSha1 also seems to have influenced, at least in part, this unusually high frequency of increased MIC values of triclosan. In fact, studies conducted by Furi et al. (2016)13 with different ST and CC isolates identified seven integration sites in only 10 isolates carrying TnSha1, indicating the absence of a preferential insertion site.13 Those authors also suggested that integration has usually occurred in hairpin-forming regions that always seem to lead to a partial deletion of the target sequence.13 However, we did not find evidence of deletions in the insertion region in the CC5 isolates analysed.

Although the mechanism described here involves an increased-MIC phenotype, a recent study using an Escherichia coli model suggested that chronic exposure to triclosan may promote selection for high-dose resistant strains.17 It is also of concern that pre-treatment of E. coli (MG1655) and MRSA (FPR3757, USA300-related strain) with clinically used concentrations of triclosan caused as much as a 10,000-fold increase in tolerance to bactericidal antibiotics in vitro. Indeed, the pre-treatment of mice with triclosan (100 μg/mL in water per 21 days) reduced ciprofloxacin efficacy up to 100-fold in a urinary infection model challenged with E. coli UTI89.15 Additionally, those authors found reduced activity of Spot hydrolase among triclosan pre-treated bacteria (E. coli MG1655), which leads to the accumulation of the alamone guanosine tetraphosphate (ppGpp), a characteristic that is frequently associated with antibiotic persisters.15 Previous studies have shown that triclosan resistance in S. aureus induces a small-colony variant (SCV) phenotype.16 SCVs represent a subpopulation of persisters...
showing prolonged lag time, reduced susceptibility to antimicrobials and increased persistence inside host cells. 

In Brazil, there is no restriction on the use of triclosan in cosmetic and personal hygiene products, and it is used in a variety of household and end-user products as an antiseptic for hand hygiene, and to decontaminate medical devices, surfaces and surgical sutures in healthcare settings. Thus, increased exposure to triclosan may explain the high frequency of increased MIC values of this biocide among the CC5 isolates analysed, the most prevalent HA-MRSA clonal complex in the Rio de Janeiro metropolitan area. 

The failure of Boye’s method for classifying TnSha1-positive MRSA as a carrier of SCC mec revealed here the important direct consequences for epidemiological studies of MRSA. For instance, CC5-SCCMecIV isolates (related to the so-called paediatric clone) have been shown to be associated with paediatric populations while CC5-SCCMecII isolates have been more associated with infections in older patients and more frequently found in blood. These two lineages also often present quite different antimicrobial susceptibility patterns, with SCC mec II isolates having increased antimicrobial resistance. Misclassification could impact further evaluation of these associations. Thus, we do not recommend the use of the primers 1272F1 and 1272R1 described in Boye’s method for differentiating SCC mec type IV from type II unless the isolates are negative for TnSha1. We strongly suggest the inclusion of more specific primers for SCC mec type II, such as KDP F1 and KDP R1, described by Oliveira and De Lencastre (2002).

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Transparency declarations
There are no conflicts of interest to declare.

Data availability
The local genome database, composed of 8324 genomes used for searching TnSha1, is available at https://data.mendeley.com/drafts/mkwvsp85rhg. The NCBI access number for the genomes analysed was cited in this work or can be obtained from the study of Viana et al., 2021.

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Supplementary data
Figure S1, Tables S1 to S3 and File S1 (Materials and methods) are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

Triclosan resistance and SCC mec misclassification

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