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ccrB typing tool: an online resource for staphylococci *ccrB* sequence typing

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Sir,

In 2006, we published a manuscript in this journal describing the allelic variation in the *ccrAB* locus in a representative collection of methicillin-resistant *Staphylococcus aureus* (MRSA) based on DNA sequencing of internal fragments of both genes.[1] This work confirmed the very close relationships among *ccrAB* alleles associated with SCC*mec* types I–IV and VI, which was found to be independent of the MRSA lineage, geographic origin or isolation period. Moreover, particularly for the *ccrB* gene, SCC*mec* types II and IV, both defined by *ccrAB* allotype 2, could be discriminated. This method provides a significant improvement in *ccrAB* typing resolution since these SCC*mec* types have different epidemiological characteristics: type II is mostly found among hospital-acquired MRSA (e.g. ST5/USA100 and ST36/EMRSA-16 clones), whereas type IV is mostly found among community-acquired MRSA (e.g. ST80, ST30 and ST1 clones). Based on these observations and since SCC*mec* types are defined based on the *ccrAB* allotype and the genetic organization of the *mecA* locus,[2] we have proposed that sequencing an internal fragment of *ccrB* could be used as a SCC*mec* typing strategy, either as a first-line assay or as a confirmation tool for SCC*mec* type assignments. From a practical perspective, *ccrB* sequence typing can be easily incorporated in other widespread sequence-based MRSA typing strategies, such as multilocus sequencing (MLST) and *spa* typing.

Under this rationale, we have developed an online resource for storage and automatic analysis of *ccrB* internal sequences obtained using our previously published protocol. The so-called '*ccrB* typing tool' was launched in late October 2007 and is freely available at http://www.ccrbtyping.net. A detailed tutorial is available online as well the contacts of the site developers. Users can access the *ccrB* typing online database either anonymously or as registered users; registration is required for submission of data to the public database or to create a personal

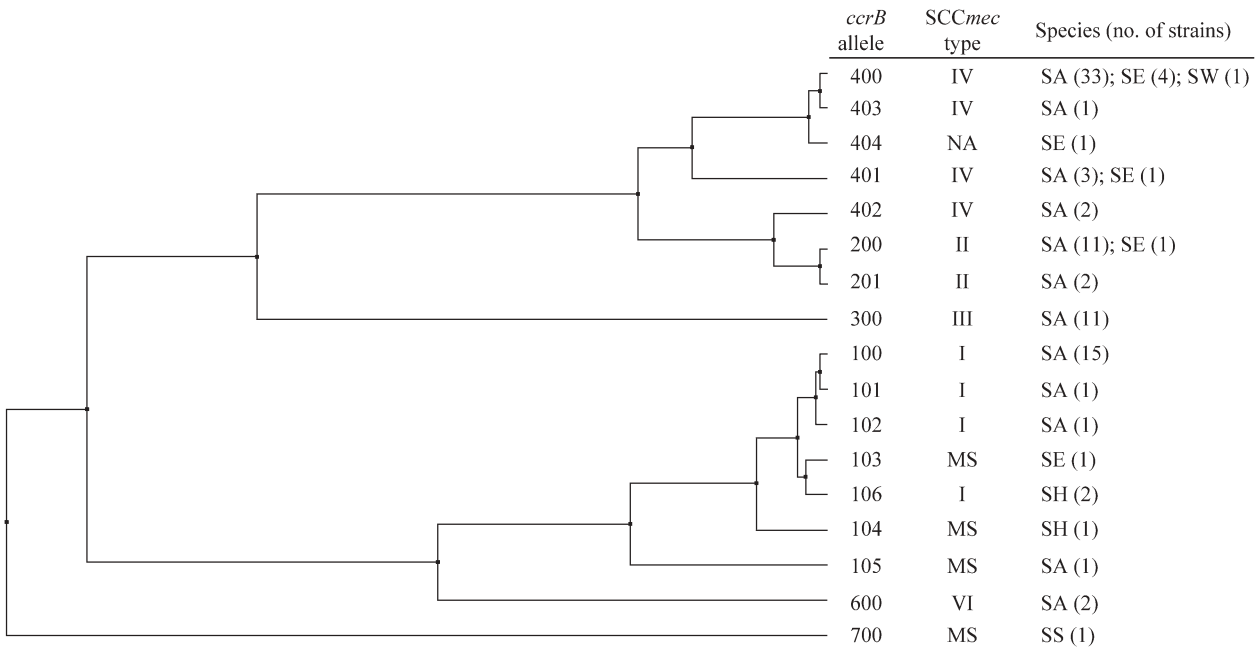

Figure 1. Average distance tree using identity percentage for all prototype sequences available on 15 November 2007 at the '*ccrB* typing tool' online resource. The tree was automatically drawn by the Java applet available through the '*ccrB* typing tool' using the default parameters. For each prototype sequence (*ccrB* allele), the species where it was described is indicated as well as, between parentheses, the number of strains of each species with that allele. SA, *S. aureus*; SE, *S. epidermidis*; SH, *S. hominis*; SS, *S. saprophyticus*; SW, *S. warneri*; NA, not available; MS, methicillin-susceptible (SCC*mec* negative).

and private online database of *ccrB* alleles. Users can paste *ccrB* internal sequences in the FASTA format, which can be automatically trimmed to fix the sequence length for analysis at 455 bp. Then, after an automatic multiple sequence alignment to the database known *ccrB* alleles,[3] the user's sequence is either assigned to a *ccrB* allele (based on a 100% homology) or to a new one, if a homology between 90% and 100% is found to any of the available alleles. If a new allele is found, the most similar allele is indicated and, after submission to the public database, an allele number is assigned. Based on this assignment, a prediction of the *ccrAB* allotype and SCC*mec* type is also outputted. The user can also check all outputs by inspecting a graphical display of the multiple sequence alignment and the reconstruction of neighbour-joining or average-distance trees available through a Java applet.[4] Users can also select subsets of private and public sequences to run the multiple sequence algorithm and visualize the resulting trees. If users choose to submit their data to the public database, the submission process is validated by a curator that checks for data consistency and quality. If a new *ccrB* allele is found, users are requested to upload both trace files.

Upon development of the '*ccrB* typing tool', we have deposited all sequences described in Oliveira *et al.*[1] and also all *ccrB* sequences available at GenBank (www.ncbi.nlm.nih.gov, last accessed on 16 November 2007) covering the same 455 bp used in the *ccrB* typing tool. Besides *ccrB* sequences for *S. aureus*, 13 sequences for coagulase-negative staphylococci (CoNS), such as *Staphylococcus epidermidis*, *Staphylococcus hominis*, *Staphylococcus saprophyticus* and *Staphylococcus warneri,* were inserted. Altogether, as of 15 November 2007, 96 *ccrB* internal sequences were made available, which were assigned to 17 alleles (Figure 1). In spite of the increased size of the collection and the extension to staphylococcal species other than *S. aureus*, the conclusions obtained for the well-defined MRSA collection are still valid. This is particularly relevant if one takes into account that 45 sequences were assigned to SCC*mec* type IV, the most variable structural type, and that there is a great diversity in the SCC*mec* elements circulating in the CoNS population.[5,6] Among the 96 *ccrB* isolates, five were described for methicillin-susceptible strains (i.e. SCC*mec* negative). Although one sequence was assigned to a new cluster (*ccrB* allele 700), the remaining sequences were clustered in previously existing groups, suggesting that *ccrB* typing might also be useful for the characterization of other SCC elements. In conclusion, *ccrB* typing is indeed a promising SCC*mec* typing strategy since there is robust correlation among *ccrB* allelic clusters and SCC*mec* types.

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Transparency declarations

None to declare.

References

1. Oliveira DC, Milheirico C, Vinga S *et al.* Assessment of allelic variation in the *ccrAB* locus in methicillin-resistant *Staphylococcus aureus* clones. *J Antimicrob Chemother* 2006; **58**: 23–30.

2. Ito T, Katayama Y, Asada K *et al.* Structural comparison of three types of staphylococcal cassette chromosome *mec* integrated in the chromosome in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2001; **45**: 1323–36.

3. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 2004; **32**: 1792–7.

4. Clamp M, Cuff J, Searle SM *et al.* The Jalview Java alignment editor. *Bioinformatics* 2004; **20**: 426–7.

5. Hanssen AM, Kjeldsen G, Sollid JU. Local variants of staphylococcal cassette chromosome *mec* in sporadic methicillin-resistant *Staphylococcus aureus* and methicillin-resistant coagulase-negative staphylococci: evidence of horizontal gene transfer? *Antimicrob Agents Chemother* 2004; **48**: 285–96.

6. Miragaia M, Thomas JC, Couto I *et al.* Inferring a population structure for *Staphylococcus epidermidis* using multilocus sequence typing data. *J Bacteriol* 2007; **189**: 2540–52.

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'Grandmother penicillin'—not in vogue, but clinically still effective

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Sir,

Although dental health in the developed world is improving, patients with acute dentoalveolar or odontogenic abscesses still present frequently at dental surgeries or emergency units.[1] Following its discovery by Fleming in 1928,[2] penicillin has long been recognized as an effective standard therapy in patients with orofacial infections. However, penicillin is no longer in favour[3] due to the more frequent isolation of resistant bacteria