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Identification of genetic variation exclusive to specific lineages associated with *Staphylococcus aureus* bacteraemia

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SUMMARY

Background: Meticillin-resistant *Staphylococcus aureus* (MRSA) bacteraemia cases have declined since 2003, and have mostly been due to two epidemic (E) strains, E15 (multilocus sequence type clonal complex CC22) and E16 (CC30). By contrast, the incidence of meticillin-susceptible *S. aureus* (MSSA) bacteraemia has remained largely unchanged and our understanding of these isolates has remained poor.

Aim: To investigate the distribution and nucleotide sequence of heterogeneous regions between successful lineages using the 2009 British Society for Antimicrobial Chemotherapy (BSAC) Bacteraemia Resistance Surveillance Programme collection of *S. aureus*.

Methods: *S. aureus* isolates ($N = 202$) comprised of 103 MRSA and 99 MSSA isolates were analysed using fluorescent amplified fragment length polymorphism (FAFLP) to detect nucleotide variations due to lineage-specific sequence motifs as well as differences in the distribution of mobile genetic elements between lineages.

Findings: E15 and E16 MRSA strains comprised 79% and 6% of the collection in 2009 respectively. Six lineages, including CC22 and CC30, were associated with MRSA bacteraemia in the UK and Ireland. MSSA isolates were more diverse with 19 different lineages detected. FAFLP revealed lineage-specific sequence variations in loci encoding factors such as proteases or factors involved in haem biosynthesis, both of which may affect the success of major *S. aureus* lineages. Proteins encoded on certain mobile genetic elements or involved in cobalamin biosynthesis were found to be exclusive to CC8, CC22, or CC30.

Conclusion: Overall, the genetic diversity among regions of the core genome and mobile genetic elements may alter antimicrobial resistance and the production of virulence or fitness factors that may be linked to strain success.

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Introduction

Staphylococcus aureus is among the top three causes of hospital and community-associated bacteraemia.[1] Cases can be difficult to treat and are associated with high rates of mortality.[2] In the UK, meticillin-resistant *S. aureus* (MRSA) bacteraemia cases increased during the 1990s, peaked in 2003 and remained at a high level through to 2006. At their peak in 2003, MRSA accounted for 42% (7659 cases) of all *S. aureus* bacteraemias, before declining to 23% by 2009.[3–5] During this period two strains predominated, epidemic (E) MRSA strains E15 and E16 (sequence type ST22 MRSA-IV and ST36 MRSA-II respectively), together accounting for 96% of MRSA bacteraemia cases.[6,7] The change in the epidemiology of strains causing MRSA bacteraemia, with the decline of E16 from 21% in 2001 to 9% in 2007, the increase of E15 from 76% in 2001 to 85% in 2007, and the decline of E15 after 2007, raises the possibility that the number of MRSA bacteraemia cases attributed to other MRSA strains may increase.

In contrast to MRSA, the molecular epidemiology of meticillin-susceptible *S. aureus* (MSSA) causing bacteraemia remains poorly understood. Since the introduction of mandatory reporting of MSSA bacteraemia in 2011, a slight increase in the number of MSSA isolates has been observed in 2013–14.[8] As the incidence of MRSA bacteraemia has declined, more attention has focused on bacteraemias due to MSSA.

The genetic diversity within *S. aureus* bacteraemias has been investigated previously using molecular tools such as multi-locus sequence typing (MLST), staphylococcal cassette chromosome *mec* (SCC*mec*) typing, amplified fragment length polymorphism (AFLP) and whole-genome sequencing.[9–14] Remarkable insights have been gained including how dominant MRSA lineages (such as clonal complex CC1, CC5, CC8, CC22, CC30, and CC59, based on MLST) have acquired an SCC*mec* element and have undergone clonal expansion. These successful lineages were defined as those lineages that have given rise to multiple epidemic strains and have spread worldwide. Whereas whole-genome sequencing has provided the ultimate level of discrimination for studies of major strains, hurdles remain (such as unified approaches to analysis, interpretation and storage of data) before it can be used routinely for the majority of laboratories.[15,16]

AFLP is a whole-genome sampling methodology which detects polymorphisms within restriction endonuclease recognition sequences in addition to insertions and deletions within core conserved and variable genomic regions.[12] Genetic analysis using AFLP not only confirms the clonal population structure of *S. aureus* revealed by MLST but also highlights the genetic heterogeneity within major lineages of *S. aureus*.[9,13,17–21] Unique AFLP markers can be identified for isolates within a specific AFLP-based cluster, as we have shown previously using fluorescent AFLP (FAFLP) for isolates of six major MRSA lineages.[22] However, as MSSA were not investigated in this study, the role of these markers in the success of *S. aureus* lineages was not determined.

In this report, a selection of the 2009 collection of *S. aureus* isolates from the British Society for Antimicrobial Chemotherapy (BSAC) Bacteraemia Resistance Surveillance Programme was examined to determine the genetic diversity among *S. aureus* causing bacteraemia in the UK and Ireland. FAFLP analysis was used to investigate the diversity and to identify distinct genetic differences between successful *S. aureus* lineages.[23] This study examined a larger number of MRSA isolates ($N = 103$) than our previous study.[22] In light of the decline of MRSA causing bacteraemia, MSSA isolates ($N = 99$) were also included in this study. Furthermore, additional approaches were used to identify variant regions between lineages. The DNA sequence of variant regions provided an insight into the functional role of these regions and their potential impact on strain success.

Methods

Bacteria, culture conditions and DNA extraction

Staphylococcus aureus isolates for the 2009 BSAC Bacteraemia Resistance Surveillance Programme were collected from 25 centres throughout the UK and Ireland.[24] Each centre could contribute a maximum of 20 consecutive isolates. The collection was comprised of 470 *S. aureus* isolates of which 107 were MRSA and 363 were MSSA. A total of 202 *S. aureus* isolates from the collection were examined. *S. aureus* positive for the *mecA* gene (103 MRSA) and a subset of 99 MSSA isolates representing the first four or five consecutive isolates from 24 centres and a single isolate from the remaining centre were selected. Isolates were cultured on Columbia agar plates supplemented with horse blood (Oxoid, Thermo Scientific, Basingstoke, UK) and incubated aerobically at 37°C for 24 h. Genomic DNA was extracted using a MagNA Pure LC Robot and MagNA Pure LC DNA Isolation Kit III (Roche, Burgess Hill, UK) according to the manufacturer's recommendations and stored at −20°C.

Isolates were characterized by performing minimum inhibitory concentrations (MICs) of a panel of antimicrobials by the BSAC agar dilution method and by determining the presence of the *mecA* gene by polymerase chain reaction (PCR) as described previously.[25–27] The panel of antimicrobials consisted of ciprofloxacin, clindamycin, daptomycin, erythromycin, fusidic acid, gentamicin, linezolid, minocycline, oxacillin, penicillin, piperacillin–tazobactam, rifampicin, teicoplanin, tetracycline, tigecycline, trimethoprim, vancomycin, and mupirocin.

S. aureus characterization

Isolates were further characterized using SCC*mec* typing and MLST. Previously described multiplex PCR assays were used to detect SCC*mec* types I–V. Isolates assigned to SCC*mec* IV were further subtyped (IVa to IVh) as described previously.[10,11] A combination of previously described multiplex PCR assays targeting specific genes within the *mec* class or *ccr* complex were performed on isolates not assigned to SCC*mec* types I–V to detect SCC*mec* types VI–XI.[28] Amplicons were resolved on a 2% agarose gel and the amplicon sizes were used to infer the SCC*mec* type and subtype. MLST of *S. aureus* isolates was performed as described previously.[9] Sequencing reactions were performed using the BigDye Terminator v1.1 Cycle Sequencing Kit and separated using an ABI 3730 automated capillary DNA sequencer (Life Technologies, Warrington, UK). The sequence of each amplicon was assigned to an allele by comparison to the MLST database (http://saureus.mlst.net/) using BioNumerics 6.1 software (Applied Maths, Sint-Martens-Latem, Belgium).[29] Isolates were assigned to clonal lineages (or clonal complexes,

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