Characterization of community and hospital *Staphylococcus aureus* isolates in Southampton, UK

S. M. Green,¹ P. Marsh,¹ N. Ahmad,¹ J. M. C. Jefferies¹,² and S. C. Clarke²,³

¹HPA RMN South East Regional Laboratory, Southampton, UK
²Molecular Microbiology Group, School of Medicine, University of Southampton, Southampton, UK
³Hampshire and Isle of Wight Health Protection Unit, Whiteley, UK

*Staphylococcus aureus* infections are a burden to healthcare systems. There remains a lack of understanding on the relative contributions of *S. aureus* infection in the healthcare and community settings. In this study, 59 *S. aureus* isolates were selected for molecular analysis. The mobile variant staphylococcal cassette chromosome mec type IV was present in both healthcare-associated meticillin-resistant *S. aureus* (HA-MRSA) and community-associated MRSA (CA-MRSA), as was the Panton–Valentine leukocidin gene. PFGE identified 24 distinct clonal groups whilst multi-locus sequence typing identified 26 different sequence types, including four with new combinations of alleles. This is the first time, to our knowledge, that a selection of CA and HA MSSA and MRSA strains have been subjected to molecular analysis and comparison in the UK. Definitions for CA-MRSA need further debate as the movement of strains between healthcare and community settings is confounding the use of epidemiological definitions.

INTRODUCTION

*Staphylococcus aureus* infections, particularly those involving meticillin-resistant strains (MRSA), are a recognized and emerging problem in healthcare. Whilst most MRSA infections in the UK are healthcare-associated (HA-MRSA), community-associated MRSA (CA-MRSA) infections in patients without HA risk factors have recently been reported (Nathwani et al., 2008). Characteristic community MRSA (C-MRSA) strains have been identified worldwide that differ phenotypically and genotypically from HA-MRSA strains and CA-MRSA of healthcare origin. Of particular note is that C-MRSA is susceptible to a wider range of antibiotics than HA-MRSA, and can produce Panton–Valentine leukocidin (PVL) (Deresinski, 2005). C-MRSA strains are thought to have evolved via meticillin-susceptible *S. aureus* (MSSA) strains acquiring the *lukS-PV* and *lukF-PV* genes for PVL production and the resulting PVL⁺ MSSA gaining meticillin resistance through integration of the smaller, more mobile staphylococcal cassette chromosome mec (SCCmec) types IV or V (Boyle-Vavra & Daum, 2007). PVL⁺ MSSA therefore represents a potential source of new C-MRSA strains. C-MRSA may display specific virulence and resistance patterns distinct from those of most circulating HA-MRSA and CA-MRSA strains and may cause significant morbidity and mortality in previously healthy individuals. The situation is complicated by the fact that hospital occurrences of C-MRSA have been reported in the UK (Otter & French, 2006). C-MRSA strains are therefore of particular interest.

METHODS

Fifty-nine *S. aureus* isolates, derived from 58 patients at Southampton University Hospitals NHS Trust or primary care centres between July 2002 and March 2007, were used. CA isolates were identified using CDC criteria: isolation within 48 h of hospitalization or from an outpatient or community healthcare centre setting with no evidence of previous hospitalization in the preceding year or previous *S. aureus* isolation (by interrogation of the hospital patient database). Isolates were selected to represent a cross-section of genetic diversity observed in *S. aureus* isolates obtained from clinical samples submitted to the Health Protection Agency Southampton Laboratory for epidemiological analysis (predominantly for outbreak investigations) during the study period (n=434). Antimicrobial susceptibilities to fluclaxocillin, erythromycin, vancomycin, teicoplanin, gentamicin, rifampicin, fusidic acid, tetracycline, trimethoprim, chloramphenicol, mupirocin, neomycin and ciprofloxacin were measured using the agar doubling dilution method (British Society of Antimicrobial Chemotherapy). All isolates were characterized by macrorestriction analysis of Smal-digested genomic DNA using the HARMONY PFGE protocol and reference collection of epidemic MRSA (EMRSA) strains (Murchan
Fig. 1. Genetic analysis of isolates. Dendrogram (UPGMA type) of PFGE profiles showing PFGE types (A–N, P–W, Y, Z) and subtypes, MLST sequence types (ST), clonal complexes (CC), MRSA SCCmec types, PVL-positive isolates and isolates meeting criteria for community acquisition (CA). PFGE types corresponding to UK EMRSA-15, 16 and 17 are indicated (E-15, E-16 and E-17, respectively). NT, Non-typable by this method. The SCCmec typing multiplex PCR included primers specific for the mecA gene as an internal positive control for meticillin resistance. Therefore, any sample with a SCCmec result (including 'NT') is mecA+. 
Isolates were grouped into unrelated PFGE clonal groups (>six band differences) and numbered subtypes (one to six bands different) (Tenover et al., 1995).

All isolates were further characterized by multi-locus sequence typing (MLST) (Enright et al., 2000) and by PVL, meca and femB, and SCCmec PCR using standard or modified methods as published previously (McDonald et al., 2005; Oliveira & de Lencastre, 2002; Zhang et al., 2005). DNA extraction was performed using standard methodology with the QIAamp DNA Mini kit (Qiagen). MLST relationships were analysed using eBURST v3. Differences between means were assessed using a two-tailed Student’s t-test.

RESULTS AND DISCUSSION

Thirteen (22 %) isolates were CA, of which 54 % were MRSA. The majority (71 %) of the CA-MRSA strains carried SCCmec-IV and three were PVL+ C-MRSA strains (the only other PVL+ MRSA being a C-MRSA HA isolate). SCCmec-IV was also present in nine PVL+ HA-MRSA and two PVL- CA-MRSA isolates. A further five PVL+ isolates were MSSA. Sixty-four per cent of HA isolates were MRSA, with SCCmec types I–V identified in this group. Three HA-MRSA isolates carried SCCmec types which did not give recognizable patterns with the multiplex PCR assays, and were thus designated non-typable by this method. These three MRSA isolates (according to the meca and femB duplex real-time PCR) nevertheless possessed the meca gene. MRSA carrying SCCmec IV or V was resistant to significantly (P<0.02) fewer antimicrobials (range 1–6, mean 3.3) than strains carrying SCCmec I, II or III (range 4–10, mean 7.2).

Clonal analysis

PFGE identified 24 distinct clonal groups (A–N, P–W, Y, Z; Fig. 1). Fourteen of these were represented by single isolates (50 % MSSA), whilst the remaining 10 comprised between one and 14 subtypes. MLST identified 26 different sequence types (STs), including four with new combinations of alleles (all MSSA). PVL was found in isolates belonging to three MRSA STs (ST8, ST30, ST80) and five

Fig. 2. eBURST of all known S. aureus STs showing the 26 STs observed in this study. eBURST analysis of all S. aureus STs at www.mlst.net. Clonal complexes of STs (represented by dots) differing at only one out of seven alleles are joined by lines. STs observed in Southampton are labelled and indicated by grey circles. Novel STs identified in this study are marked with an asterisk (*).
MSSA STs (ST1, ST15, ST121, ST291, ST1413). Fifty percent of ST30 isolates were PVL+. These two strains carried SCCmec-IV and resembled the C-MRSA South West Pacific (SWP) clone by PFGE. Retrospective examination of the Southampton PFGE database identified a further five SWP-like strains (isolated between February 2003 and February 2007) which all carried SCCmec-IV and were PVL+ (data not shown). Two of the seven SWP clone-like isolates were from CA-MRSA cases.

**Analysis of MLST data using eBURST**

eBURST analysis of the 59 strains indicated that the 26 STs split into six clonal complexes (CCs) and 12 singletons STs (Fig. 2). ST22 was the predominant ST (n=16). The majority of STs present as more than one isolate were represented by both CA and HA isolates, with the exception of ST123 (n=2), ST247 (n=2) and ST15 (n=4), which only occurred as HA isolates. ST239 and related STs 8 and 241 formed a CC made up only of MRSA isolates, whereas all other CCs contained both MRSA and MSSA isolates. Data from the current study were compared to the entire *S. aureus* MLST database using comparative eBURST (data not shown). Thirteen out of the 26 STs present were predicted founders of CCs containing globally prevalent isolates. This indicates that the STs present in Southampton have not recently evolved but are representatives of well-known clones. Four further STs were subfounders. A subfounder is defined as a single locus variant (SLV) of the main founder linked to a group of its own SLVs, which are in turn double locus variants of the main founder.

This is the first time, to our knowledge, that a selection of CA and HA MSSA and MRSA strains have been subjected to molecular analysis and comparison in the UK. In our study, the mobile variant SCCmec-IV was present in both HA-MRSA and CA-MRSA. The presence of PVL+ CA-MSSA and HA-MSSA isolates suggests a potential risk of mecA gene transfer from mobile SCCmec-containing MRSA to PVL+ MSSA, or the phage-borne transfer of lukS-PV and lukF-PV from PVL+ MSSA or MRSA to PVL− MRSA. We found MRSA strains with STs associated with both healthcare (ST22, ST239) and community (ST8, ST30) infections in isolates that were epidemiologically defined as HA and CA. HA infections caused by C-MRSA have been previously reported (Otter & French, 2006) and CA infections may be the result of transmission of HA strains in the community setting by a recently hospitalized patient. Our results suggest that two-way movement of *S. aureus* strains between healthcare and community settings is occurring. HA and CA PVL+ ST30-MRSA-IV isolates were found that resembled the SWP clone. A further five SWP clone-like strains were identified (one CA, all ST30-MRSA-IV, PVL+), suggesting that this C-MRSA strain has been circulating in Southampton since at least 2003. One CA ST80-MRSA-IV isolate (the European C-MRSA clone) was identified as resistant to five antimicrobials. It is known that MRSA isolates belonging to this clone are widespread in Europe, Asia and the Middle East (Deurenberg & Stobberingh, 2008; Larsen et al., 2008; Tristan et al., 2007) and that along with other lineages of MRSA, resistant isolates of this clone have been previously reported (Ellington et al., 2010). Therefore, the opportunities for such isolates to acquire resistance determinants may arise in community or hospital settings. Attempting to make a distinction between HA and CA strains is becoming increasingly difficult. There is no agreed definition for CA-MRSA and the movement of strains between healthcare and community settings is confounding the use of epidemiological definitions. This underlines the importance of molecular characterization to identify the potentially more virulent C-MRSA strains, rather than relying on epidemiological data alone.

**ACKNOWLEDGEMENTS**

This study was funded by a Health Protection Agency Pump Priming Fund grant awarded to P.M., S.M.G. and S.C.C.

**REFERENCES**


