Epidemiology of community MRSA obtained from the UK West Midlands region.

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Running title: *Community MRSA from the West Midlands.*

Summary

Between January 2005 and December 2005, 199 meticillin-resistant *Staphylococcus aureus* (MRSA) isolates were obtained from non-hospitalized patients presenting skin and soft tissue infections to local general practitioners. The study area incorporated 57 surgeries from three Primary Care Trusts in the Lichfield, Tamworth, Burntwood, North and East Birmingham regions of Central England, UK. Following antibiotic sensitivity testing, pulsed-field gel electrophoresis, Panton-Valentine leukocidin gene detection and SCC*mec* element assignment, 95% of the isolate population were determined genetically related to hospital epidemic strains EMRSA-15 and EMRSA-16. In total 87% of the isolate population harboured SCC*mec* IV, 9% SCC*mec* II and 4% were identified to carry novel SCC*mec* IIIa^{-mecI}. When mapped to patient home postcode a diverse distribution of isolates harbouring SCC*mec* II and SCC*mec* IV was observed, however the majority of isolates harbouring SCC*mec* IIIa^{-mecI} were isolated from patients residing in the north-west of the study region highlighting a possible localized clonal group. Evidential transmission of MRSA from the hospital setting into the surrounding community population, as demonstrated by this study, warrants the need for targeted patient screening and de-colonisation in both the clinical and community environment.

Key words: *EMRSA-15, EMRSA-16, MRSA, SCCmec, PFGE, community.*

Introduction

Meticillin-resistant *Staphylococcus aureus* and its association with UK hospital-acquired infections has been well documented.[1-4] However, following four pediatric deaths in Dakota and Minnesota,[5] the emergence of MRSA in the community setting has become a major focal point of epidemiological research.[6-11] Meticillin-resistant *Staphylococcus aureus* clones of true community origin arise from the horizontal transfer of *mecA* into circulating meticillin-sensitive *Staphylococcus aureus* strains and are typically characterized by the presence of SCC*mec* IV, susceptibility to non-ß-lactams and the gene locus for Panton-Valentine leukocidin (PVL).[6, 12-13] Predominantly associated with invasive soft-tissue infections, community-derived MRSA strains are evidently a worldwide concern.[8] However, increasing evidence for the dissemination of hospital epidemic strains into the community population indicate that the lines defining hospital- and community- MRSA acquisition are becoming increasingly difficult to define.[14-15] In the UK the prevalence of true community-acquired MRSA (CA-MRSA) remains low. However, the detection of *pvl* genes in strains related to EMRSA-15 and 16 clones [16] and the report of *pvl*-positive CA-MRSA in the West Midlands [17] warrants continued surveillance for emerging virulent clones within the UK clinical and community population. The aim of this study was to characterize the epidemiology of MRSA strains circulating within the Central England community encompassing the regions of Lichfield, Tamworth, Burntwood, North and East Birmingham.

Methods

Bacterial isolates

Between January 2005 and December 2005, 199 MRSA isolates were obtained from non-hospitalized patients presenting skin and soft tissue infections to local general practitioners. The study area incorporated 57 surgeries from three Primary Care Trusts in the Lichfield, Tamworth, Burntwood, North and East Birmingham regions of Central England, UK. Prior in-patient or out-patient hospital history was determined from the Good Hope Hospital information system (dating back to 1994). Patient GP records were consulted in those cases where hospital records were not available. Permission to undertake this study was granted by the Medical Director of Good Hope Hospital NHS Trust and the Director of Public Health of North Birmingham Primary Care Trust. Formal ethical approval was not required as patient identifiers, such as name and NHS registration number, were deleted from the datasets.

Antibiotic sensitivity testing

Isolate identification was undertaken at the Good Hope Hospital NHS Trust, Sutton Coldfield, following standard laboratory procedures. For all presumptive MRSA, sensitivity to erythromycin, trimethoprim, rifampicin, gentamicin, tetracycline, mupirocin, vancomycin, fusidic acid and meticillin was determined using the British Society for Antimicrobial Chemotherapy (BSAC) disc diffusion method.[18]

Molecular characterization of MRSA

Rapid DNA extraction by boiling

For all PCR protocols employed, chromosomal DNA was extracted by a rapid boil extraction method.[19]

Multiplex PCR for SCC*mec* element assignment

Staphylococcal Cassette Chromosome *mec* (SCC*mec*) element assignment and Panton-Valentine leukocidin gene locus detection were determined for all MRSA isolates using primers and cycle conditions obtained from previously described methods.[6, 20] All PCR protocols were undertaken using Gene Amp PCR system 9700 (Applied Biosystems, UK). In addition, SCC*mec* types were mapped to the centroids

of the full UK unit postcodes (each UK postcode is shared by 15-20 households) determined by the UK National Statistics Postcode Directory.

Pulsed-field gel electrophoresis

Pulsed-field gel electrophoresis was performed on all MRSA isolates using previously published protocols.[21-22] Staphylococcus *aureus* NCTC 8325 was used as a control and molecular weight marker.

Results

Patient demographics

Within the population of this study, 33 (17%) patients were recorded to be in long term nursing care facilities and 166 (83%) lived at home. Additionally, 148 (74%) patients were 65 years old and above and 51 (26%) were below the age of 65. In total, 38% of the patient study set had no recorded inpatient or outpatient hospital visit.

Antibiotic sensitivity testing

Seven MRSA isolates exhibited resistance to six or more antibiotics, five of which harboured SCC*mec* II and two SCC*mec* IV. Isolates with resistance to six or more antibiotics were designated multi-drug resistant (MR-MRSA). Thirty-seven MRSA isolates were resistant to meticillin alone, 36 of which harboured SCC*mec* IV and one harboured SCC*mec* II. The remaining 155 isolates exhibited resistance to at least one non-ß-lactam (Table I).

Multiplex PCR for SCC*mec* element assignment

Following SCC*mec* element assignment, 173 (87%) of the MRSA isolate population harboured SCCmec IV, 18 (9%) harboured SCC*mec* II and eight (4%) were identified to carry a novel variant designated SCC*mec* IIIa^{-mecI}. Variant SCC*mec* IIIa^{-mecI} was characterized by the amplification of the loci specific to SCC*mec* IIIa but minus the 209bp amplification product internal to *mecI*.[20] Mapping SCC*mec* type to patient home postcode demonstrated a diverse distribution of isolates harbouring SCC*mec* II and SCC*mec* IV. Seven of the eight isolates harbouring SCC*mec* IIIa^{-mecI} appeared to be localized in the north-west of the study region (figure 1).

Pulsed-field gel electrophoresis analysis

Pulsed-field gel electrophoresis analysis of the 199 MRSA isolates identified five distinct PFGE profile groups designated A1, B1, C1, D1 and E1. Profile A1 was identified in 21% of the isolate population and represented a typical EMRSA-15 progenitor profile.[23] Following Tenover criteria,[21] 20 closely related subtypes were designated to profile A1 (A2-A21) and all isolates belonging to this group were identified to carry SCC*mec* IV. In total 173 isolates were genetically related subtypes of EMRSA-15 accounting for 87% of the isolate population. PFGE profile B1 was identified in 2% of the isolate population and represented a typical EMRSA-16 progenitor profile.[24] Five closely related subtypes were designated to profile B1 (B2-B6) and all isolates belonging to this group were identified to carry SCC*mec* II. In total 16 isolates were genetically related subtypes of EMRSA-16 accounting for 8% of the isolate population. Two SCC*mec* II isolates were characterized to PFGE profiles C1 and D1 and were not related to EMRSA-16 by Tenover criteria. One closely related subtype was designated to profile E1 (E2) and all eight isolates belonging to this group were identified to carry SCC*mec* IIIa^{-mecI}.

Pvl gene locus detection

The *pvl* gene locus was not detected in any of the 199 MRSA isolates analysed in this investigation.

Discussion

Following PFGE analysis, 95% of the isolates retrieved from the Lichfield, Tamworth, Burntwood, North and East Birmingham community, were representative of hospital acquired EMRSA-15 or EMRSA-16 genetic lineage.[23-24] In addition 81% of the isolate population displayed resistance to at least one non-ß-lactam and the gene locus for Panton-Valentine leukocidin was not detected in any of the 199 MRSA isolates, further exemplifying the probable acquisition of these strains from a clinical setting.[25-26] In contrast, within the USA, community acquired-MRSA (USA 300) has become a significant strain both in the community and hospital environment.[27] Following phenotypic and genotypic analysis, all MRSA isolates retrieved from the West Midlands community in this study were not characteristic of CA-MRSA, further highlighting MRSA isolates from the UK community predominantly represent a spillover of epidemic MRSA strains from the hospital setting.[28] This observed epidemiological pattern is similar to that reported in West Midlands based hospital studies where the predominant strains also relate to EMRSA-15 and EMRSA-16 PFGE profiles.[19]

Patients predisposed to MRSA may remain undetected if symptoms fail to present whilst undergoing hospital treatment. The subsequent discharge of carrier patients

may provide an ideal mechanism for transmission of nosocomial derived strains within the community population.[29] Additionally, re-admittance of such carrier patients may further impact upon current hospital MRSA rates.[30-31] It is interesting to note that 17% of the patient population were resident in a nursing care facility and 74% were above the age of 65. Such patients are typically associated with higher rates of MRSA carriage,[32-33] however, 38% of the patient study set had no recorded inpatient or outpatient hospital visit. As 96% of isolates from these patients were genetically related to EMRSA 15 or 16, this observation may further reflect the spread of nosocomial MRSA within the community setting from those patients susceptible to MRSA carriage to individuals without recent recorded hospital contact. With this in mind, hospital infection control measures should therefore act upon reducing the reservoir of hospital-associated MRSA in the general population. Such action should take into account the discharge of symptomatic and asymptomatic MRSA positive patients and the implementation of targeted screening in patients with associated risk factors for acquisition (in particular the elderly).[14, 29, 32] It should, however, be recognized that it was not possible to estimate the exposure of these patients to other hospital settings with the information available in this study.

Previous studies have associated SCC*mec* IV with increased fitness and transmissibility.[34-35] It should therefore be noted that 87% of the MRSA isolate population harboured SCC*mec* IV, providing further evidence for the importance of this genetic element in MRSA transmission outside of the nosocomial setting.

Molecular analysis of novel SCC*mec* IIIa^{-mecI}

From the map produced in figure 1, a diverse distribution of SCC*mec* II and SCC*mec* IV was observed. In contrast, seven of the eight isolates harbouring SCC*mec* IIIa^{-mecI} appeared to be localized in the north-west of the study region. Following PFGE analysis, isolates harbouring SCC*mec* IIIa^{-mecI} produced banding profiles that were 95% related and genetically distinct from the remaining isolate population. This may possibly signify a population of minor sporadic clones,[36] unfortunately ethical considerations prevented detailed investigation into patient demographics which may have provided scope for further analysis of the SCC*mec* IIIa^{-mecI} cluster. Novel SCC*mec* elements are reportably widespread [20, 37] however, identification of SCC*mec* IIIa^{-mecI} was not unique to this study setting.[38] The continued evolution of novel structural variants currently complicates the accurate identification of SCC*mec* and the future development of a robust typing protocol is currently required.[39-42]

In conclusion, following phenotypic and genotypic analysis, all MRSA isolates retrieved from the West Midlands community were not characteristic of CA-MRSA. The majority of the isolate population was identified to be related to hospital epidemic strains, demonstrating the probable transmission of MRSA from the hospital setting into the surrounding community population. The predominance of MRSA isolates harboring SCC*mec* IV demonstrates the transmissible success of SCC*mec* IV to prevail outside of the hospital setting. Furthermore the identification of

novel SCC*mec* types reveals the genetic plasticity of the SCC*mec* structure and the potential for emerging minor sporadic clones. The UK Department of Health has committed itself to comprehensive hospital based MRSA screening; however this strategy does not take into account the possibility of MRSA transmission within the community setting. The migration of hospital acquired MRSA strains into the community environment raises questions about the best way to control the organism, but these questions are beyond the scope of this paper. However, this study has shown that detailed molecular investigation is feasible and advantageous in understanding MRSA in the community. Similar studies, coupled with more detailed patient data, could be valuable in the assessment of hospital-based or community-based interventions designed to control MRSA.

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References

1 Johnson AP, Aucken HM, Cavendish S, *et al*. Dominance of EMRSA-15 and -16 among MRSA causing nosocomial bacteraemia in the UK: analysis of isolates from the European Antimicrobial Resistance Surveillance System (EARSS). *J Antimicrob Chemother* 2001; **48:** 143-144.
2 Johnson AP, Pearson A, Duckworth G. Surveillance and epidemiology of MRSA bacteraemia in the UK. *J Antimicrob Chemother* 2005; **56:** 455-462.
3 Wyllie DH, Crook DW, Peto TE. Mortality after Staphylococcus aureus bacteraemia in two hospitals in Oxfordshire, 1997-2003: cohort study. *Bmj* 2006; **333:** 281.
4 Das I, O'Connell N, Lambert P. Epidemiology, clinical and laboratory characteristics of Staphylococcus aureus bacteraemia in a university hospital in UK. *J Hosp Infect* 2007; **65:** 117-123.
5 Anon. Four pediatric deaths from community-acquired methicillin-resistant Staphylococcus aureus-Minnesota and North Dakota. *MMWR* 1999; **48:** 707-710.
6 Lina G, Piemont Y, Godail-Gamot F, *et al*. Involvement of Panton-Valentine leukocidin-producing Staphylococcus aureus in primary skin infections and pneumonia. *Clin Infect Dis* 1999; **29:** 1128-1132.
7 Okuma K, Iwakawa K, Turnidge JD, *et al*. Dissemination of new methicillin-resistant Staphylococcus aureus clones in the community. *J Clin Microbiol* 2002; **40:** 4289-4294.
8 Vandenesch F, Naimi T, Enright MC, *et al*. Community-acquired methicillin-resistant Staphylococcus aureus carrying Panton-Valentine leukocidin genes: worldwide emergence. *Emerg Infect Dis* 2003; **9:** 978-984.
9 Rossney A, Morgan P, O'Connell B. Community-acquired PVL+ MRSA in Ireland: a preliminary report. *Euro Surveill* 2005; **10:** E050421.1.
10 King MD, Humphrey BJ, Wang YF, Kourbatova EV, Ray SM, Blumberg HM. Emergence of community-acquired methicillin-resistant Staphylococcus aureus USA 300 clone as the predominant cause of skin and soft-tissue infections. *Ann Intern Med* 2006; **144:** 309-317.
11 Kluytmans-Vandenbergh MF, Kluytmans JA. Community-acquired methicillin-resistant Staphylococcus aureus: current perspectives. *Clin Microbiol Infect* 2006; **12:** 697-698.
12 Baba T, Takeuchi F, Kuroda M, *et al*. Genome and virulence determinants of high virulence community-acquired MRSA. *Lancet* 2002; **359:** 1819-1827.
13 Tenover FC, McDougal LK, Goering RV, *et al*. Characterization of a strain of community-associated methicillin-resistant Staphylococcus aureus widely disseminated in the United States. *J Clin Microbiol* 2006; **44:** 108-118.
14 Salgado CD, Farr BM, Calfee DP. Community-acquired methicillin-resistant Staphylococcus aureus: a meta-analysis of prevalence and risk factors. *Clin Infect Dis* 2003; **36:** 131-139.
15 Adedeji A, Weller TM, Gray JW. MRSA in children presenting to hospitals in Birmingham, UK. *J Hosp Infect* 2007; **65:** 29-34.
16 Holmes A, Ganner M, McGuane S, Pitt TL, Cookson BD, Kearns AM *Staphylococcus aureus* Isolates Carrying Panton-Valentine Leucocidin Genes in England and Wales: Frequency, Characterization, and Association with Clinical Disease. *J Clin Microbiol* 2005; **43:** 2384-2390.
17 Health Protection Agency. Hospital-associated transmission of Panton-Valentine

leukocidin (PVL) positive community-associated MRSA in the West Midlands. *Comm Dis Rep CDR Wkly* 2006; **16**.
18 Andrews JM. The development of the BSAC standardized method of disc diffusion testing. *J Antimicrob Chemother* 2001: **48:** 29-42.
19 Caddick JM, Hilton AC, Rollason J, Lambert PA, Worthington T, Elliott TS. Molecular analysis of methicillin-resistant Staphylococcus aureus reveals an absence of plasmid DNA in multidrug-resistant isolates. *FEMS Immunol Med Microbiol* 2005; **44:** 297-302.
20 Oliveira DC, Lencastre H. Multiplex PCR strategy for rapid identification of structural types and variants of the mec element in methicillin-resistant Staphylococcus aureus. *Antimicrob Agents Chemother* 2002; **46:** 2155-2161.
21 Tenover FC, Arbeit RD, Goering RV, *et al*. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995; **33:** 2233-2239.
22 Murchan S, Kaufmann ME, Deplano A, *et al.* Harmonization of pulsed-field gel electrophoresis protocols for epidemiological typing of strains of methicillin-resistant Staphylococcus aureus: a single approach developed by consensus in 10 European laboratories and its application for tracing the spread of related strains. *J Clin Microbiol* 2003; **41:** 1574-1585.
23 O'Neill GL, Murchan S, Gil-Setas A, Aucken HM. Identification and characterization of phage variants of a strain of epidemic methicillin-resistant Staphylococcus aureus (EMRSA-15). *J Clin Microbiol* 2001; **39:** 1540-1548.
24 Murchan S, Aucken HM, O'Neill GL, *et al*. Emergence, spread, and characterization of phage variants of epidemic methicillin-resistant Staphylococcus aureus 16 in England and Wales. *J Clin Microbiol* 2004; **42:** 5154-5160.
25 Livermore DM. Antibiotic resistance in staphylococci. *Int J Antimicrob Agents* 2000; **16:** Suppl 1 S3-S10.
26 Rice LB. Antimicrobial resistance in gram-positive bacteria. *Am J Med* 2006; **34:** Suppl 1 S11-S19.
27 Patel M, Kumar RA, Stamm AM, Hoesley CJ, Moser SA, Waites KB. USA300 Genotype Community-Associated Methicillin-Resistant *Staphylococcus aureus* as a Cause of Surgical Site Infections. *J Clin Microbiol* 2007; **45:** 3431-3433.
28 Bygott J, Enoch DA, Carson RP, Karas JA. Presumed community-acquired meticillin-resistant Staphylococcus aureus (MRSA) isolates reflect spillover of healthcare-associated MRSA. *J Hosp Infect* 2008. doi:10.1016/j.jhin.2008.02.002
29 Warshawsky B, Hussain Z, Gregson DB, *et al*. Hospital- and community-based surveillance of methicillin-resistant Staphylococcus aureus: previous hospitalization is the major risk factor. *Infect Control Hosp Epidemiol* 2000; **21:** 724-727.
30 Karas JA, Enoch DA, Emery MM. Community-onset healthcare-associated MRSA bacteraemia in a district general hospital. *J Hosp Infect* 2006; **62:** 480-486.
31 Robotham JV, Scarff CA, Jenkins DR, Medley GF. Meticillin-resistant Staphylococcus aureus (MRSA) in hospitals and the community: model predictions based on the UK situation . *J Hosp Infect* 2007; **65:** 93-99.
32 Morgan M, Evans-Williams D, Salmon R, Hosein I, Looker DN, Howard A. The population impact of MRSA in a country: the national survey of MRSA in Wales, 1997. *J Hosp Infect* 2000; **44:** 227-239
33 Barr B, Wilcox MH, Brady A, Parnell P, Darby B, Tompkins D. Prevalence of methicillin-resistant Staphylococcus aureus colonization among older residents of care homes in the United Kingdom. *Infect Control Hosp Epidemiol* 2007; **28**: 853-859.

34 Ma XX, Ito T, Tiensasitorn C, *et al*. Novel type of staphylococcal cassette chromosome mec identified in community-acquired methicillin-resistant Staphylococcus aureus strains. *Antimicrob Agents Chemother* 2002; **46:** 1147-1152.
35 Daum RS, Ito T, Hiramatsu K, *et al*. A novel methicillin-resistance cassette in community-acquired methicillin-resistant Staphylococcus aureus isolates of diverse genetic backgrounds. *J Infect Dis* 2002; **186:** 1344-1347.
36 Aires de Sousa M, de Lencastre H. Evolution of sporadic isolates of methicillin-resistant Staphylococcus aureus (MRSA) in hospitals and their similarities to isolates of community-acquired MRSA. *J Clin Microbiol* 2003; **41:** 3806-3815.
37 Shore A, Rossney AS, Keane CT, Enright MC, Coleman DC. Seven novel variants of the staphylococcal chromosomal cassette mec in methicillin-resistant Staphylococcus aureus isolates from Ireland. *Antimicrob Agents Chemother* 2005; **49:** 2070-2083.
38 Lu PL, Chin LC, Peng CF, *et al*. Risk factors and molecular analysis of community methicillin-resistant Staphylococcus aureus carriage. *J Clin Microbiol* 2005; **43:** 132-139.
39 Zhang K, McClure JA, Elsayed S, Louie T, Conly JM. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome mec types I to V in methicillin-resistant Staphylococcus aureus. *J Clin Microbiol* 2005; **43:** 5026-5033.
40 Kondo Y, Ito T, Ma XX, *et al*. Combination of multiplex PCRs for staphylococcal cassette chromosome mec type assignment: rapid identification system for mec, ccr, and major differences in junkyard regions. *Antimicrob Agents Chemother* 2007; **51:** 264-274.
41 Boye K, Bartels MD, Andersen IS, Møller JA, Westh H. A new multiplex PCR for easy screening of methicillin-resistant *Staphylococcus aureus* SCC*mec* types I-V. *Clin Microbiol Infect* 2007; **13:** 725-727.
42 Milheiriço C, Oliveira DC, de Lencastre H. Update to the multiplex PCR strategy for assignment of mec element types in Staphylococcus aureus. *Antimicrob Agents Chemother* 2007; **51:** 3374-3377.

Legends

Table I.
Antibiotic resistance phenotypes and SCC*mec* types of 199 MRSA isolates obtained from the Lichfield, Tamworth, Burntwood, North and East Birmingham community.

Figure 1.
SCC*mec* types mapped to patient home postcode for 199 MRSA isolates obtained from the Lichfield, Tamworth, Burntwood, North and East Birmingham community.