Prevalence and characterization of human mecC methicillin-resistant Staphylococcus aureus isolates in England

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Objectives: There are limited data available on the epidemiology and prevalence of methicillin-resistant Staphylococcus aureus (MRSA) in the human population that encode the recently described mecA homologue, mecC. To address this knowledge gap we undertook a prospective prevalence study in England to determine the prevalence of mecC among MRSA isolates.

Patients and methods: Three hundred and thirty-five sequential MRSA isolates from individual patients were collected from each of six clinical microbiology laboratories in England during 2011–12. These were tested by PCR or genome sequencing to differentiate those encoding mecA and mecC. mecC-positive isolates were further characterized by multilocus sequence typing, spa typing, antimicrobial susceptibility profile and detection of PBP2a using commercially available kits.

Results: Nine out of the 2010 MRSA isolates tested were mecC positive, indicating a prevalence among MRSA in England of 0.45% (95% CI 0.24%–0.85%). The remainder were mecA positive. Eight out of these nine mecC MRSA isolates belonged to clonal complex 130, the other being sequence type 425. Resistance to non-β-lactam antibiotics was rare among these mecC MRSA isolates and all were phenotypically identified as MRSA using oxacillin and cefoxitin according to BSAC disc diffusion methodology. However, all nine mecC isolates gave a negative result using three different commercial PBP2a detection assays.

Conclusions: mecC MRSA are currently rare among MRSA isolated from humans in England and this study provides an important baseline prevalence rate to monitor future changes, which may be important given the increasing prevalence of mecC MRSA reported in Denmark.

Keywords: MRSA, mec genes, S. aureus, surveillance

Introduction

Staphylococcus aureus is a versatile, opportunistic pathogen able to cause a wide range of diseases in humans, from minor skin infections to severe illnesses such as sepsis, toxic shock, endocarditis and pneumonia. It is also able to colonize and infect a variety of other host species, including farm and companion animals and wildlife. The emergence and dissemination of methicillin-resistant S. aureus (MRSA) since the early 1960s has posed a major challenge to the treatment of S. aureus infections. Methicillin resistance in S. aureus is conferred by the acquisition of one of several staphylococcal cassette chromosome mec (SCCmec) elements, which carry the mecA gene encoding a penicillin-binding protein homologue (PBP2a) with reduced affinity for β-lactam antibiotics. We identified a novel mecA homologue, mecA_GA251, encoded in a new SCCmec element, designated type XI, among human and bovine MRSA isolates in the UK and Denmark. This mecA homologue, subsequently named mecC, exhibits only 69% identity at the DNA level and 63% identity at the protein level to the previously described mecA/PBP2a. As a result, it is not detectable by routine mecA-specific PCR approaches or PBP2a slide agglutination tests. mecC MRSA have now been isolated in small numbers from humans and a wide range of other host species in several European countries: Republic of Ireland, France, Sweden, the
Netherlands, Germany, Austria, Switzerland, Finland, Spain, Norway and Belgium. However, the origin and epidemiology of these strains are poorly understood and there are limited data on their prevalence. Importantly, the frequency of mecC MRSA has increased significantly in Denmark since 2003.19

To provide baseline data for future surveillance in the UK, we undertook a prospective survey of a total of 2010 MRSA isolates collected from six clinical microbiology laboratories in England and screened these by PCR or genome sequencing for mecA and mecC.

Methods

Isolate collection and assessment of mec gene status

Three hundred and thirty-five sequential MRSA isolates from individual patients were identified according to local procedures from screening and clinical samples at five hospital clinical microbiology laboratories from October 2011 to August 2012 (Table 1). These were sent to Cambridge for PCR detection of mecA and mecC, as described previously.17 These were isolates drawn from hospitals and other healthcare providers in the catchment area of each laboratory, including community-based general practitioners. Methicillin resistance was based on phenotypic resistance (cefoxitin disc diffusion, Vitek 2 or chromogenic agars) in all cases and not on molecular detection of mecA or PBP2a. Isolates from a sixth hospital (Addenbrooke’s Hospital, Cambridge; Table 1) were collected as above and genome sequenced as part of an independent study. These were not assessed by PCR but by interrogation of their genome sequences using BLAST analysis to identify mecA and mecC MRSA isolates with confirmation of the presence of femB as a species marker of S. aureus. The analysis of 2010 isolates provides the power to detect mecC MRSA prevalence at a lower limit of 0.05% at the 95% confidence level.

Antimicrobial susceptibility testing and slide agglutination for PBP2a

All mecC MRSA isolates were analysed using the Vitek 2 system (bioMérieux, Basingstoke, UK). In brief, suspensions of cultures were made in 0.45% sodium solution from growth on Columbia blood agar, adjusted to a turbidity equivalent to that of a 0.5 McFarland standard and used to load the test cards, which were used in accordance with the manufacturer’s instructions. The Staph AST-P620 card was automatically filled, sealed and inserted into the Vitek 2 reader–incubator module (incubation temperature 37°C), and fluorescence measurements were performed every 15 min for up to 18 h. Cefoxitin and oxacillin resistances were also assayed by disc diffusion following BSAC guidelines (version 11.1 May 2012) and the MICs of cefoxitin and oxacillin were determined using Etest strips (bioMérieux). All mecC MRSA isolates identified were tested with three commercially available PBP2a detection assays according to the manufacturers’ instructions: the Mastalex® MRSA Test (Mast Diagnostics, Bootle, UK), the Penicillin Binding Protein (PBP2) Latex Agglutination Test (Oxoid, Basingstoke, UK) and the Alere™ PBP2a Culture Colony Test (Alere Ltd, Stockport, UK). The mecA-positive MRSA strain NCTC12493 was used as a positive control.

Genome sequencing and spa typing

All mecC MRSA isolates underwent whole genome sequencing using the HiSeq2000 platform (Illumina, Little Chesterford, UK) to confirm their mecC gene status and determine their multilocus sequence type (ST). Isolates that were PCR negative for either mecA or mecC were also genome sequenced to confirm their mec gene status. The species identity of isolates negative by PCR for femB was tested by assessing their growth and morphology on Staph Brilliance 24 and MRSA Brilliance 2 agar plates (both Oxoid) and by PCR to detect nuc.10 spa typing was performed using the primers spo-1113f (5′-TAA AGA CGA TCC TTC GGT GAG C-3′) and spo-1514r (5′-CAG CAG TAG TGC CGT TTG CTT-3′) as described by Ridom GmbH (Würzburg, Germany).

Results and discussion

PCR (or genome sequence analysis in the case of Addenbrooke’s Hospital, Cambridge) revealed that 9 isolates out of a total of 2010 MRSA collected were mecC MRSA. These mecC MRSA isolates were largely from screening samples (six isolates), but included three isolates from skin and soft tissue infections. The remaining MRSA isolates were all mecA positive, which provides a prevalence rate of mecC MRSA among all MRSA collected of 0.45% with a 95% CI of 0.24%–0.85%. All 2010 isolates were confirmed to be S. aureus. In the majority of cases this identification was based on the presence of femB as detected by PCR or genome sequencing. However, 12 out of the 1675 isolates (0.72%) tested by PCR for femB were negative for an amplicon and were instead confirmed to be S. aureus based on their growth on Staph Brilliance and MRSA Brilliance agar plates and all were positive for nuc. The basis for the negative femB PCR result is under investigation and may relate to divergence in the femB primer binding sites. Indeed, a small number of S. aureus isolates negative for femB using alternative PCR approaches have been reported previously.21,22 Two isolates were negative by PCR for both mecA and mecC, but genome sequencing revealed that they were indeed mecA positive and carried previously described mecA genes (NCBI accession numbers FJ390057 and AF411935) with divergence in the primer binding sites used in this study.

Genome sequencing confirmed that each isolate positive for mecC by PCR encoded mecC within an SCCmec type XI. Multilocus ST derived from the genome sequences revealed five different STs among the nine isolates, including a novel ST, ST2574. Eight of the isolates belonged to clonal complex (CC) 130, with the remaining isolate belonging to ST425. Five spa types were represented:
t843, t6220, t9280, t11702 and t11706 (Table 2). All mecC MRSA isolates were resistant to cefoxitin and oxacillin using BSAC guidelines for disc diffusion, while MICs varied from 8 to 32 mg/L for oxacillin and from 8 to 16 mg/L for cefoxitin (Table 2). Antimicrobial susceptibility testing using Vitek 2 revealed that resistance to non-β-lactam antibiotics was rare, the only example being a single isolate, Ta222, displaying resistance to erythromycin and inducible resistance to clindamycin (Table 2). All nine isolates displayed the unusual Vitek 2 resistance profile of being resistant to cefoxitin, but susceptible to oxacillin. This feature of mecC MRSA, likely caused by structural differences between the mecA- and mecC-encoded PBP2a, has been described previously and may be helpful in the identification of mecC MRSA isolates. The susceptibility to oxacillin seen using Vitek 2 is in disagreement with our oxacillin disc diffusion results. mecC-encoded PBP2a has been shown to be less stable at 37°C than at 30°C, which may explain this discrepancy, oxacillin disc diffusion being performed at 30°C, but Vitek 2 analysis at 37°C. Cefoxitin resistance is presumably still seen using Vitek 2, even at 37°C, because of the higher affinity mecC-encoded PBP2a has for cefoxitin versus oxacillin. All nine mecC MRSA isolates gave negative results when assayed with three different commercial PBP2a slide agglutination assays, confirming the difficulty of detecting mecC MRSA using this approach.

This is the first formal prospective prevalence study of mecC MRSA performed in the UK and these data provide a baseline prevalence for the future surveillance of mecC MRSA in England. Continued monitoring of mecC is potentially important given the increase in prevalence of mecC MRSA reported in Denmark. There are few other data on mecC MRSA prevalence elsewhere, but in Germany a large multicentre prospective study identified a single mecC isolate among 1604 tested in 2004–05 and again a single isolate from 1603 tested in 2010–11. This indicates a prevalence of 0.06% with no change between the study periods. In contrast, the prevalence in Denmark was both higher and increasing, rising from 1.91% in 2010 to 2.78% in 2011. A survey of 565 human MRSA isolates in Switzerland failed to find any mecC MRSA, indicating that the prevalence there is lower than in Denmark. Clearly, there are significant and as yet unexplained differences in mecC MRSA prevalence between different countries, and the recent increase reported in Denmark suggests that it would be prudent to monitor prevalence in the UK and elsewhere.

None of the hospitals used oxacillin to identify MRSA, which has been shown to be less reliable than cefoxitin for the detection of mecC MRSA. Nonetheless, it is possible that some mecC MRSA may have been missed during primary isolation. For instance, small numbers of mecC MRSA isolates grow poorly on MRSA-selective agars, presumably due to their having low cefoxitin/oxacillin MIC values. An area for future study may be the comparison and standardization of primary isolation methods in relation to mecC MRSA.

The majority of mecC MRSA isolates found in our survey belonged to CC130, which agrees with the data of Garcia-Alvarez et al. showing that CC130 was the most common lineage among their retrospective testing for mecC MRSA among human isolates in the UK and Denmark. Both CC130 and ST425 are the predominant lineages among mecC MRSA isolates found not only in humans but also in other host species elsewhere, and genome sequencing has provided strong evidence of cross-species transmission of mecC MRSA between humans and livestock. Of the five spa types recovered in this study, neither t11702 nor t11706 appear to have been reported previously among mecC MRSA, whilst the other three, t843, t6220 and t9280, have. There were multiple CCs belonging to the same spa type and multiple spa types within the same CC, illustrating the difficulty of inferring CC from spa type data.

As reported for mecC MRSA isolated elsewhere in Europe and from different host species, resistance to non-β-lactam antibiotics was uncommon among these English mecC MRSA isolates. The origins of mecC MRSA and SCCmec type XI are unclear, but mecC has also been detected in Staphylococcus stepanovicii and Staphylococcus xylosus and Staphylococcus sciuri. This suggests a possible origin for mecC in coagulase-negative staphylococci, as proposed for mecA and clinical microbiology laboratories should therefore be aware not only of mecC MRSA but of the possible occurrence of mecC in other pathogenic species of methicillin-resistant staphylococci.
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References
1 Ito T, Hiramatsu K, Tomas A et al. Guidelines for reporting novel mecA gene homologues. Antimicrob Agents Chemother 2012; 56: 4997–9.
2 Garcia-Alvarez L, Holden MTG, Lindsay H et al. Methicillin-resistant Staphylococcus aureus with a novel mecA homologue in human and bovine populations in the UK and Denmark: a descriptive study. Lancet Infect Dis 2011; 11: 595–603.
3 Shore AC, Deasy EC, Slickers P et al. Detection of staphylococcal cassette chromosome mec type XI carrying highly divergent mecA, mecC, mecR1, blaz, and ccr genes in clinical isolates of clonal complex 130 methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother 2011; 55: 3765–73.
4 Laurent F, Chardon H, Høenni M et al. MRSA harboring mecA variant gene mecC, France. Emerg Infect Dis 2012; 18: 1465–7.
5 Hellman J, Olsson-Liljequist B. A Report on Swedish Antibiotic Utilisation and Resistance in Human Medicine. Solna: Swedish Institute for Communicable Disease Control, 2012.
6 Unnerstad HE, Bengtsson B, Rantzien MH et al. Methicillin-resistant Staphylococcus aureus containing mecC in Swedish dairy cows. Acta Vet Scand 2013; 55: 6.
7 Moncke S, Gavier-Widen D, Mattsson R et al. Detection of mecC-positive Staphylococcus aureus (CC130-MRSA-XI) in diseased European hedgehogs (Erinaceus europaeus) in Sweden. PLoS One 2013; 8: e66166.
8 Sabat AJ, Koksal M, Akkerboom V et al. Detection of new methicillin-resistant Staphylococcus aureus strains that carry a novel genetic homologue and important virulence determinants. J Clin Microbiol 2012; 50: 3374–7.
9 Cuny C, Layer F, Strommenger B et al. Rare occurrence of methicillin-resistant Staphylococcus aureus CC130 with a novel mecA homologue in horses in Germany. PLoS One 2011; 6: e24360.
10 Schaumburg F, Koeck R, Mellmann A et al. Population dynamics among methicillin-resistant Staphylococcus aureus isolates in Germany during a 6-year period. J Clin Microbiol 2012; 50: 3186–92.
11 Walther B, Wieler LH, Vincze S et al. MRSA variant in companion animals. Emerg Infect Dis 2012; 18: 2017–20.
12 Loncaric I, Kubber-Heiss A, Posautz A et al. Characterization of methicillin-resistant Staphylococcus spp. carrying the mecC gene, isolated from wildlife. J Antimicrob Chemother 2013; 14: 2222–5.
13 Bassett P, Prod’homme G, Senn L et al. Very low prevalence of methicillin-resistant Staphylococcus aureus carrying the mecC gene in western Switzerland. J Hosp Infect 2013; 83: 259–9.
14 Gindonis V, Tapanen S, Myllyniemi AL et al. Occurrence and characterization of methicillin-resistant staphylococci from bovine mastitis milk samples in Finland. Acta Vet Scand 2013; 55: 61.
15 Garcia-Garrote F, Cercenado E, Marin M et al. Methicillin-resistant Staphylococcus aureus carrying the mecC gene: emergence in Spain and report of a fatal case of bacteraemia. J Antimicrob Chemother 2014; 69: 45–50.
16 Medhus A, Slettemeas JS, Marstein L et al. Methicillin-resistant Staphylococcus aureus with the novel mecC gene variant isolated from a cat suffering from chronic conjunctivitis. J Antimicrob Chemother 2013; 68: 968–9.
17 Paterson GK, Larsen AR, Robb A et al. The newly described mecA homologue, mecC_{GA251}, is present in methicillin-resistant Staphylococcus aureus isolates from a diverse range of host species. J Antimicrob Chemother 2012; 67: 2809–13.
18 Vandendriessche S, Vanderhaeghen W, Soares FV et al. Prevalence, risk factors and genotypic diversity of methicillin-resistant Staphylococcus aureus carried by humans and animals across livestock production sectors. J Antimicrob Chemother 2013; 68: 1510–6.
19 Paterson A, Stegger M, Helbig M et al. Epidemiology of methicillin-resistant Staphylococcus aureus carrying the novel mecC gene in Denmark corroborates a zoonotic reservoir with transmission to humans. Clin Microbiol Infect 2013; 19: E16–22.
20 Brakstad OG, Aasbaek K, Maeland JA. Detection of Staphylococcus aureus by polymerase chain reaction amplification of the nuc gene. J Clin Microbiol 1992; 30: 1654–60.
21 Mohanasundaram KM, Laliha MK. Comparison of phenotypic versus genotypic methods in the detection of methicillin resistance in Staphylococcus aureus. Indian J Med Res 2008; 127: 78–84.
22 Kobayashi N, Wu H, Kojima K et al. Detection of mecA, femA, and femB genes in clinical strains of staphylococci using polymerase chain reaction. Epidemiol Infect 1994; 113: 259–66.
23 Kim C, Milheirico C, Gardete S et al. Properties of a novel PBP2A protein homolog from Staphylococcus aureus strain LGA251 and its contribution to the β-lactam-resistant phenotype. J Biol Chem 2012; 287: 36854–63.
24 Cartwright EJP, Paterson GK, Raven KE et al. Use of Vitek 2 antimicrobial susceptibility profile to identify mecC in methicillin-resistant Staphylococcus aureus. J Clin Microbiol 2013; 51: 2732–4.
25 Skov R, Larsen AR, Kears A et al. Phenotypic detection of mecC-MRSA: cefoxitin is more reliable than oxacillin. J Antimicrob Chemother 2014; 69: 133–5.
26 Harrison EM, Paterson GK, Holden MTG et al. Whole genome sequencing identifies zoonotic transmission of MRSA isolates with the novel mecA homologue mecC. EMBO Mol Med 2013; 5: 509–15.
27 Barraud O, Laurent F, Francois B et al. Severe human bone infection due to methicillin-resistant Staphylococcus aureus carrying the novel mecC variant. J Antimicrob Chemother 2013; 68: 2949–50.
28 Harrison EM, Paterson GK, Holden MTG et al. A Staphylococcus xylosus isolate with a new mecA allele type. Antimicrob Agents Chemother 2013; 57: 1524–8.
29 Harrison EM, Paterson GK, Holden MTG et al. A novel hybrid SCCmec-mecC region in Staphylococcus sciuri. J Antimicrob Chemother 2014; 69: 911–8.
30 Couto I, de Lancastre H, Severina E et al. Ubiquitous presence of a mecA homologue in natural isolates of Staphylococcus sciuri. Microb Drug Resist 1996; 2: 377–91.
31 Tsukibishita S, Kuwahara-Arai K, Sasaki T et al. Origin and molecular evolution of the determinant of methicillin resistance in staphylococci. Antimicrob Agents Chemother 2010; 54: 4352–9.