Prevalence and properties of mecC methicillin-resistant Staphylococcus aureus (MRSA) in bovine bulk tank milk in Great Britain

G. K. Paterson1*, F. J. E. Morgan1, E. M. Harrison1, S. J. Peacock2–5, J. Parkhill5, R. N. Zadoks6,7 and M. A. Holmes1

1Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge CB3 0ES, UK; 2Health Protection Agency, Microbiology Services Division Cambridge, Level 6 Addenbrooke’s Hospital, Hills Road, Cambridge CB2 0QQ, UK; 3Department of Medicine, University of Cambridge, Addenbrooke’s Hospital, Cambridge CB2 0QQ, UK; 4Cambridge University Hospitals NHS Foundation Trust, Cambridge CB2 0QQ, UK; 5The Wellcome Trust Sanger Institute, Wellcome Trust, Genome Campus, Cambridge CB10 1SA, UK; 6Moredun Research Institute, Bush Loan, Penicuik EH26 0PZ, UK; 7Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, 464 Bearsden Road, Glasgow G61 1QH, UK

*Corresponding author. Tel: +44-(0)1223-337636; Fax: +44-(0)1223-337610; E-mail: gkp27@cam.ac.uk

Received 13 August 2013; returned 5 September 2013; revised 20 September 2013; accepted 25 September 2013

Objectives: mecC methicillin-resistant Staphylococcus aureus (MRSA) represent a newly recognized form of MRSA, distinguished by the possession of a divergent mecA homologue, mecC. The first isolate to be identified came from bovine milk, but there are few data on the prevalence of mecC MRSA among dairy cattle. The aim of this study was to conduct a prevalence study of mecC MRSA among dairy farms in Great Britain.

Methods: Test farms were randomly selected by random order generation and bulk tank samples were tested for the presence of mecC MRSA by broth enrichment and plating onto chromogenic agar. All MRSA isolated were screened by PCR for mecA and mecC, and mecC MRSA were further characterized by multilocus sequence typing, spa typing and antimicrobial susceptibility testing.

Results: mecC MRSA were detected on 10 of 465 dairy farms sampled in England and Wales (prevalence 2.15%, 95% CI 1.17%–3.91%), but not from 625 farms sampled in Scotland (95% CI of prevalence 0%–0.61%). Seven isolates belonged to sequence type (ST) 425, while the other three belonged to clonal complex 130. Resistance to non-β-lactam antibiotics was uncommon. All 10 isolates produced a negative result by slide agglutination for penicillin-binding protein 2a. mecA MRSA ST398 was detected on one farm in England.

Conclusions: mecC MRSA is widely distributed among dairy farms in Great Britain, but this distribution is not uniform across the whole country. These results provide an important baseline dataset to monitor the epidemiology of this emerging form of MRSA.

Keywords: bovine mastitis, antibiotic resistance, molecular epidemiology

Introduction

Methicillin-resistant Staphylococcus aureus (MRSA) encoding a divergent mecA homologue within a novel SCCmec type XI element were first reported in bovine and human isolates from the UK, Denmark and Eire in 2011.1,2 Originally named mecA3-MRSA, and subsequently designated mecC,3 this homologue shares 69% nucleotide identity with mecA and produces a negative result in mecA-based PCR assays and slide agglutination tests for penicillin-binding protein (PBP) 2a.

As a result of its recent discovery and these diagnostic difficulties, there are relatively few data on the prevalence and epidemiology of mecC MRSA. However, mecC MRSA isolates have now been reported from a number of additional European countries, including France,4 Finland,5 Sweden,6 the Netherlands,7–9 Austria,10 Switzerland,11 Norway12 and Belgium,13,14 and from a broad range of host species, encompassing livestock, companion animals and wildlife, including sheep,13,15 domestic cat,12,16 domestic dog,13,16 brown rat,13 hare,13 rabbit,13 otter,13 hedgehog,16 guinea pig,16 common seal13 and chaffinch.13 Furthermore, zoonotic transmission from livestock to humans in Denmark has been corroborated by epidemiological follow-up and whole genome sequencing.17,18 mecC has also been detected in other species of staphylococci, specifically Staphylococcus xylosus from bovine mastitis19 and Staphylococcus stepanovicii from a wild European lynx.10

Given that the original mecC MRSA isolate was found in bovine milk and that S. aureus is an important cause of bovine mastitis,20 we undertook a prevalence study of mecC MRSA in dairy bulk tank samples in Great Britain (GB) collected during 2011–12.
Materials and methods

Bulk tank milk samples and processing

Randomly selected bulk tank milk samples were supplied between November 2011 and October 2012 by National Milk Laboratories Ltd (Chippenham, UK), a commercial milk testing company responsible for >95% of quality assurance testing of bulk tank milk from GB dairy farms. The dairy farms to be sampled were selected by taking a list of all farms using National Milk Laboratories Ltd and placing these in random order using Microsoft Excel (Seattle, WA, USA), with the first 500 selected for sampling.

Samples from England and Wales were treated together as these are collected and processed by one laboratory, with Scottish samples processed at another. Of the 500 selected farms in England and Wales, 35 had ceased production of milk before they could be tested, and so were removed from the study, leaving a sample size of 465 dairy farms. In Scotland, the same process was used except the sampling continued beyond the first 500 farms when no mecC MRSA-positive farms were discovered in any of these, enlarging the final sample size to 625 dairy farms to increase the confidence limits of the result.

The bulk tank milk samples were collected aseptically by trained technicians for quality assurance purposes and stored at 4°C for up to 5 days before freezing at −20°C prior to testing. Samples were thawed at 37°C and 1 mL of milk was added to 4 mL of Mueller–Hinton broth (Oxoid, Basingstoke, UK) supplemented with 6.5% (w/v) NaCl. After incubation at 37°C with shaking at 200 rpm, 50 μL of culture was spread onto MRSA Brillance 2 plates (Oxoid) and incubated at 37°C for 24 h. Experiments with spiked milk indicated a lower limit of detection for mecC MRSA of ≤50 cfu/mL of milk. Potential MRSA colonies (blue colour) were subcultured on Staph Brillance 24 plates (Oxoid) and subsequently screened for mecA, mecC and femB by multiplex PCR as described previously. All mecC MRSA isolates were genome sequenced to confirm them as mecC positive, and the multilocus sequence type (ST) was derived from the genome sequence.

Antibiotic susceptibility

Resistance to oxacillin, cefoxitin, chloramphenicol, ciprofloxacin, tetracycline, erythromycin, mupirocin, clindamycin, trimethoprim, gentamicin, linezolid, penicillin, fusidic acid, neomycin and rifampicin was assessed by disc diffusion. The minimum inhibitory concentrations (MICs) of oxacillin were determined using the Etest (bioMérieux, Basingstoke, UK).

Slide agglutination for PBP2a

Bovine mecC MRSA isolates were tested with three commercially available PBP2a detection assays: the Mostelex MRSA Test (MAST, Bootle, UK), the Penicillin Binding Protein (PBP2a) Latex Agglutination Test (Oxoid) and the Alere PBP2a Culture Colony Test, according to the manufacturers' instructions. The mecA-positive strain NCTC 12493 was used as a positive control.

Results

Prevalence of mecC MRSA among GB dairy farm bulk milk

From England and Wales, 10 dairy farms from a total of 465 sampled farms were positive for mecC MRSA (Figure 1). This represents a prevalence rate of 2.15% (95% CI 1.17%–3.91%). None of the positive farms was in Wales (n = 90), giving a prevalence of 0% (95% CI 0%–4.09%), and providing a prevalence of 2.67% (95% CI 1.46%–4.84%) for the 375 dairy farms sampled in England. mecC MRSA-positive farms were found in five counties distributed from the south-west (Devon and Cornwall) to the north of England (Northumberland) (Figure 1). None of the original 500 samples from Scotland was positive for mecC MRSA and so an additional 125 samples were tested. None of these 625 Scottish samples was positive for mecC MRSA (95% CI 0%–0.61%). A comparison of the England and Wales prevalence with the Scotland prevalence using Fisher’s exact test rejected the null hypothesis of ‘no difference between the two rates’ with P = 0.0002.

Characterization of bovine mecC MRSA

Seven of the ten mecC MRSA isolates belonged to ST425, among which three spa types were represented (t6292, t1742 and t6300). The three remaining isolates belonged to clonal complex (CC) 130, two of which were ST130 and the third was ST2573, a novel yqiL single locus variant of ST130. All three CC130 isolates belonged to spa type t843. All 10 isolates were resistant to cefoxitin by disc diffusion with the Etest showing MICs varying from 6 mg/L to 12 mg/L. The MIC of oxacillin was more variable, ranging from 6 mg/L to 48 mg/L, and all isolates were also resistant to oxacillin as determined using disc diffusion.

Prevalence and characteristics of mecA MRSA from GB bulk milk

In addition to being screened for mecC, putative MRSA from the prevalence study samples were also screened by PCR for mecA. This identified a single farm in Worcestershire, England that was positive for mecA MRSA (Figure 1) and gives a prevalence in England of 0.27% (95% CI 0.05%–1.50%). This isolate belonged to ST398.

Discussion

Here we report the first prevalence study of mecC MRSA on GB dairy farms. mecC MRSA was present on 10 farms of 465 in England and Wales, but was not present on 625 farms sampled in Scotland. The absence of previous data and the recent increase in mecC MRSA isolation from humans in Denmark indicate the importance of monitoring of mecC MRSA prevalence. The data reported here provide a valuable baseline for the future surveillance of emergent mecC MRSA and show that it is already widespread among the English dairy herd.

Although most mecC MRSA-positive farms were in the south-west of England, positive farms were also recorded elsewhere, indicating that mecC MRSA among English dairy farms is not localized to one particular region. Nevertheless, a degree of regional clustering of STs and spa types was observed, e.g. both isolates in North Yorkshire were ST130, t843 and four of five isolates in Devon were ST425, t6292. The cause of such patterns is not yet clear and is under investigation, but may be the result of local cattle movements and/or shared local services.
Figure 1. Map of GB dairy farms sampled for MRSA. All sampled farms are shown; 1079 farms were MRSA negative, 10 farms were meC MRSA positive and 1 farm was meA MRSA positive. Multilocus ST, spa type and additional antibiotic resistance are indicated for meC MRSA.
The difference in prevalence between Scotland and England is statistically significant, although the reasons for this are not obvious, especially as mecC MRSA has been isolated from other host species in Scotland – including humans and wildlife.1,13 While no survey samples from Wales were positive for mecC, MRSA testing of additional milk samples that were not part of the formal prevalence study found two dairy farms in Wales (Wrexham and Gwynedd) positive for mecC MRSA (data not shown).

The majority of bovine isolates found in this study belonged to ST425, with the others belonging to CC130, including ST2573 – a novel single locus variant of ST130. Although mecC MRSA has been isolated from bovine mastitis previously,1,21 the current study examined bulk tank milk samples and so the association of these isolates with clinical disease is unclear. Interestingly, of the mecC MRSA isolates identified by Garcia-Alvarez et al.1 from clinical bovine mastitis, the majority (11/13) belonged to CC130, in contrast to the majority of isolates from bulk tank milk samples in the present study (7/10), which belonged to ST425, as did the first mecC MRSA isolated by Garcia-Alvarez et al.1 This observation may suggest that there are differences in the propensity of mecC MRSA lineages to cause clinical bovine mastitis, with CC130 more likely to cause clinical disease than ST425. Indeed, an association between colonization site and ST has been demonstrated previously for certain bovine S. aureus STs,22 and ST425 may be colonizing the teat skin rather than tissue within the mammary gland. A small number of mecC MRSA isolates have been isolated from dairy cattle in Belgium,14 Denmark17 and Sweden,23 and belonged to ST130 (four isolates), ST425 (one isolate) and ST2508 (one isolate). Similar to human mecC MRSA isolates, it appears therefore that CC130 and ST425 are also the predominant mecC MRSA lineages in dairy cattle.1

The presence of mecC MRSA in dairy cattle may represent a zoonotic risk to humans, and two case studies using traditional epidemiology and genome sequencing have identified possible zoonotic transmission between livestock and humans.3,17,18 To assess this risk we conducted a prevalence survey of mecC MRSA nasal colonization among delegates at a British cattle veterinarian conference in 2011.24 However, none was positive for mecC MRSA, suggesting a prevalence of <1% in this population based on the sample size.24 An investigation of the phylogenetic relationships between the human and bovine isolates described here and previously1 is currently underway using whole genome sequencing.

Based on disc diffusion and MIC determination by the Etest, all 10 isolates were resistant to both cefoxitin and oxacillin and therefore were phenotypically MRSA. However, all 10 strains produced a negative result using three different commercially available PBP2a assays. Variable results using these assays have been reported using human isolates1,2,24 and our data show that these assays are not likely to be useful for the diagnosis of bovine mecC MRSA strains either.

In addition to mecC MRSA, the multiplex PCR also allowed the identification of mecA MRSA, and a single mecA MRSA-positive dairy farm was identified. The prevalence rates reported here suggest mecA MRSA is considerably less frequent on GB dairy farms than mecC MRSA. This mecA isolate belonged to ST398 and, along with bulk tank ST398 isolates from four other UK dairy farms, was reported previously as the first detection of livestock-associated CC398 MRSA in the UK dairy herd.25

We have presented the first prevalence study of mecC MRSA on GB dairy farms. These data provide a valuable baseline dataset for the future surveillance of this emerging veterinary and human pathogen.

Acknowledgements
We gratefully thank National Milk Laboratories Ltd for their assistance in providing bulk tank milk samples.

Funding
This work was supported by a Medical Research Council Partnership Grant (G100178/7/1) held between the Department of Veterinary Medicine, University of Cambridge (M. A. H.), the School of Clinical Medicine, University of Cambridge (S. J. P.), the Moredun Research Institute (R. N. Z.) and the Wellcome Trust Sanger Institute (J. P. and S. J. P.).

Transparency declarations
Competing interests: none to declare.

The funder had no role in the study design, data collection, analysis, decision to publish, or preparation of the manuscript.

References
1 Garcia-Alvarez L, Holden MTG, Lindsay H et al. Meticillin-resistant Staphylococcus aureus with a novel mecA homologue. Lancet Infect Dis 2011; 11: 595–603.
2 Shore AC, Deasy EC, Slickers P et al. Detection of staphylococcal cassette chromosome mec type XI carrying highly divergent mecA, mecR1, blaZ, and ccr genes in human clinical isolates of clonal complex 130 meticillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother 2011; 55: 3765–73.
3 Ito T, Hiramatsu K, Tomasz A et al. Guidelines for reporting novel mecA gene homologues. Antimicrob Agents Chemother 2012; 6: 4997–9.
4 Laurent F, Chardon H, Haenni M et al. MRSA harboring mecA variant gene mecF. Emerg Infect Dis 2012; 18: 1465–7.
5 Gindonnis V, Taponen S, Myllyniemi AL et al. Occurrence and characterization of meticillin-resistant staphylococci from bovine mastitis milk samples in Finland. Acta Vet Scand 2013; 55: 61.
6 Monecke S, Gavriel-Wiener 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.
7 Sabat AJ, Koksal M, Akkerboom V et al. Detection of new meticillin-resistant Staphylococcus aureus strains that carry a novel genetic homologue and important virulence determinants. J Clin Microbiol 2012; 50: 3374–7.
8 Curry C, Layer F, Strommenger B et al. Rare occurrence of meticillin-resistant Staphylococcus aureus CC130 with a novel mecA homologue in humans in Germany. PLoS One 2011; 6: e24360.
9 Schaumburg F, Koeck R, Mollmann A et al. Population dynamics among meticillin-resistant Staphylococcus aureus isolates in Germany during a 6-year period. J Clin Microbiol 2012; 50: 3186–92.
10 Loncaric I, Kueber-Heiss A, Posautz A et al. Characterization of meticillin-resistant Staphylococcus spp. carrying the mecC gene, isolated from isolated. J Antimicrob Chemother 2013; 68: 2222–5.
11 Basset P, Prod’hom G, Senn L et al. Very low prevalence of meticillin-resistant Staphylococcus aureus carrying the mecC gene in western Switzerland. J Hosp Infect 2013; 83: 257–9.
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.

Paterson GK, Larsen AR, Robb A et al. The newly described mecA homologue, mecA_LGA251, is present in methicillin-resistant Staphylococcus aureus isolates from a diverse range of host species. J Antimicrob Chemother 2012; 67: 2809–13.

Vandendriessche S, Vanderhaeghen W, Soares FV et al. Prevalence, risk factors and genetic diversity of methicillin-resistant Staphylococcus aureus carried by humans and animals across livestock production sectors. J Antimicrob Chemother 2013; 68: 1510–6.

Kriegeskorte A, Ballhausen B, Idelevich EA et al. Human MRSA isolates with novel genetic homolog, Germany. Emerg Infect Dis 2012; 18: 1016–8.

Walther B, Wieler LH, Vincze S et al. MRSA variant in companion animals. Emerg Infect Dis 2012; 18: 2017–20.

Petersen A, Stegger M, Heltberg O 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.

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.

Harrison EM, Paterson GK, Holden MTG et al. A Staphylococcus xylosus isolate with a new mecC allotype. Antimicrob Agents Chemother 2013; 57: 1524–8.

Bradley AJ, Leach KA, Breen JE et al. Survey of the incidence and aetiology of mastitis on dairy farms in England and Wales. Vet Rec 2007; 160: 253–7.

Garcia-Graells C, Antoine J, Larsen J et al. Livestock veterinarians at high risk of acquiring methicillin-resistant Staphylococcus aureus ST398. Epidemiol Infect 2012; 140: 383–9.

Smith EM, Green LE, Medley GF et al. Multilocus sequence typing of intercontinental bovine Staphylococcus aureus isolates. J Clin Microbiol 2005; 43: 4737–43.

Unnerstad HE, Bengtsson B, Horn af Rantzien MH et al. Methicillin-resistant Staphylococcus aureus containing mecC in Swedish dairy cows. Acta Vet Scand 2013; 55: 6.

Paterson GK, Harrison EM, Craven EF et al. Incidence and characterisation of methicillin-resistant Staphylococcus aureus (MRSA) from nasal colonisation in participants attending a cattle veterinary conference in the UK. PLoS One 2013; 8: e68463.

Paterson GK, Larsen J, Harrison EM et al. First detection of livestock-associated meticillin-resistant Staphylococcus aureus CC398 in bulk tank milk in the United Kingdom, January to July 2012. Euro Surveill 2012; 17: pii=20337.