

Impact case study (REF3b)

Institution: University of Cambridge
Unit of Assessment: UoA6
Title of case study: A new MRSA emerging in human and bovine populations
<p>1. Summary of the impact (indicative maximum 100 words)</p> <p>Research led by Dr Holmes has identified a novel variant of methicillin-resistant <i>Staphylococcus aureus</i> (MRSA) in livestock. This represents a previously unidentified reservoir of infection which has had impact on the epidemiology of MRSA and its management. This research also impacts on antibiotic use in agriculture and its role in the emergence of antibiotic resistance. As a consequence of these research findings commercial tests and testing protocols have been developed to detect the new MRSA variant, which are now used widely in clinical settings throughout Europe. The discovery has also been used to inform policy decisions at a governmental level in the USA and Europe.</p>
<p>2. Underpinning research (indicative maximum 500 words)</p> <p><i>Staphylococcus aureus</i> causes a wide range of diseases in humans and other animals, including bovine mastitis, a very common and economically significant disease of dairy herds. MRSA was first identified in 1961 and is distinguished by the fact that it contains a <i>mecA</i> gene, which encodes a penicillin-binding protein (PBP2a) with low affinity for β-lactam antibiotics and thus confers resistance to these antibiotics, which include methicillin. The <i>mecA</i> gene is located on a mobile staphylococcal cassette chromosome (SCC) forming <i>SCCmec</i>. MRSA can be sub-classified as hospital acquired (HA-MRSA), community acquired (CA-MRSA) or livestock associated (LA-MRSA); the last emerged in the early 2000s and provides a reservoir of infection for both farm animals and humans.</p> <p>From 2006 to 2010 Dr Mark Holmes (Senior Lecturer, Department of Veterinary Medicine from 1991 to the present) conducted a project, jointly funded by Defra and HEFCE, investigating the epidemiology of bovine mastitis. This research identified a novel isolate of <i>S. aureus</i>, which was phenotypically resistant to β-lactam antibiotics at levels that would normally identify it as MRSA despite testing negative for <i>mecA</i> or PBP2a using established tests¹.</p> <p>Further research, led by Dr Holmes, culminated in whole genome sequencing of this isolate (in collaboration with the Wellcome Trust Sanger Institute) which found that the genetic basis for the β-lactam resistant phenotype was a novel <i>mecA</i> gene¹, provisionally named <i>mecA</i>_{LGA251} but since reclassified as <i>mecC</i>. <i>mecC</i> was located on a novel <i>SCCmec</i> (type XI). The failure to detect <i>mecC</i> using PCR was due to the low sequence conservation between <i>mecA</i> and <i>mecC</i>, with only 60% identity at the DNA level and 63% similarity at the amino acid level. PCR primers were designed by Holmes in 2010 to enable detection of this new MRSA.</p> <p>Following the design of these new primers, a further 13 <i>mecC</i> MRSA isolates were found in a collection of 940 <i>S. aureus</i> isolates from 465 UK cattle herds which had submitted mastitic milk samples to the UK Animal Health Veterinary Laboratories Agency (AHVLA)¹. The AHVLA had already identified 24 of these isolates that had high levels of antibiotic resistance consistent with MRSA status, but were not identified as MRSA using established PCR assays (using specific primers for <i>mecA</i>) and a standard slide latex agglutination test (using a monoclonal antibody specific for the <i>mecA</i>-encoded PBP2a)¹. These two tests were being used by the Health Protection Agency and other MRSA testing laboratories as 'gold standard' tests for MRSA at the time but they did not identify the <i>mecC</i> allele.</p> <p>Screening of <i>mecA</i>-negative MRSA isolated from humans from clinical disease and from MRSA screening, and from Scotland, England and Denmark, undertaken in 2010-11, in collaboration with national MRSA reference laboratories, identified a further 51 <i>mecC</i> isolates (still called <i>mecA</i>_{LGA251} in the literature at this time)¹. Strain typing of human and bovine isolates revealed an apparent spatial clustering, where isolates from the same geographical region were likely to share the same multi-locus sequence type (ST) or spa-type. All the isolates obtained from human samples had animal-associated STs, or were part of a clonal complex whose founder was animal-associated</p>

Impact case study (REF3b)

(predominantly bovine). In Denmark all MRSA isolates are submitted to the national reference laboratory and screening of these by the Holmes group revealed a statistically significant increase in human isolates carrying *mecC* between 2008 and 2011¹.

Research by the group into the origins and distribution of *mecC* has revealed additional homologues in coagulase-negative staphylococci; including *S. xylosus* and *S. sciuri* of animal origin⁴.

Research has been conducted by the group in collaboration with international MRSA reference laboratories to develop and validate MRSA testing protocols^{2,3,5}.

Whole genome sequencing has been performed by the group to provide evidence of the direction of transmission of MRSA carrying *mecC*. SNP analysis indicated that zoonotic transmission was likely to have occurred on two farms in Denmark⁶.

3. References to the research (indicative maximum of six references)

1. García-Álvarez, L., Holden, M. T., Lindsay, H., Webb, C. R., Brown, D. F., Curran, M. D., Holmes, M. A. (2011, August). 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*, 11(8), 595-603. doi:10.1016/S1473-3099(11)70126-8
2. Stegger, M., Andersson, P.A., Kearns, A., Pichon, B., Holmes, M.A. ... (2011 November). Rapid Detection, Differentiation and Typing of Methicillin-Resistant *Staphylococcus aureus* Harbours either *mecA* or the new *mecA* Homologue *mecA*_{LGA251}. *Clinical microbiology and infection* 2012 Apr;18(4):395-400. doi: 10.1111/j.1469-0691.2011.03715.x.
3. Pichon, B., Hill, R., Laurent, F., Rhod Larsen, A, Skov, R.L., Holmes, M.A. ... Kearns, A. Development of real-time quadruplex PCR assay for simultaneous detection of *nuc*, Pantone-Valentine Leucocidin (PVL), *mecA* and homologue *mecA*_{LGA251} *Journal of Antimicrobial Chemotherapy* 2012 Oct;67(10):2338-41. doi: 10.1093/jac/dks221.
4. Harrison EM, Paterson GK, Holden MTG, Morgan FEJ, Rhod Larsen A, Leroy S, Zadoks RN, Peacock SJ, Parkhill J, Holmes MA. A *Staphylococcus xylosus* isolate with a new *mecC* allotype. *Antimicrobial Agents and Chemotherapy* 2013 Mar;57(3):1524-8. doi: 10.1128/AAC.01882-12.
5. Becker K, Larsen AR, Skov RL, Paterson GK, Holmes MA, Sabat AJ, Friedrich AW, Köck R, Peters G, Kriegeskorte A. Evaluation of a Modular Multiplex-PCR Methicillin-Resistant *Staphylococcus aureus* Detection Assay Adapted for *mecC* Detection. *J Clin Microbiol*. 2013 Jun;51(6):1917-9. doi: 10.1128/JCM.00075-13.
6. Harrison EM, Paterson GK, Holden MT, Larsen J, Stegger M, Larsen AR, Petersen A, Skov RL, Christensen JM, Bak Zeuthen A, Heltberg O, Harris SR, Zadoks RN, Parkhill J, Peacock SJ, Holmes MA. Whole genome sequencing identifies zoonotic transmission of MRSA isolates with the novel *mecA* homologue *mecC*. *EMBO Mol Med*. 2013 Apr;5(4):509-15. doi: 10.1002/emmm.201202413.

Selected research grant support (Holmes as PI)

- Investigation of a novel *Staphylococcus aureus* isolate. Defra project grant (OD3020). 01/09/09–30/09/10. £71,207
- Partnership to investigate the emergence of MRSA clones in cattle and their transmission to man. Medical Research Council Partnership Grant. (G1001787) 01/04/011–31/03/14. £1,500,161

4. Details of the impact (indicative maximum 750 words)

Publication of the discovery of *mecC* in 2011 raised important questions about the detection and confirmation of MRSA in humans, and resulting decisions about patient care.

Prior to the research in this study, patients presenting with *mecC*-positive *S. aureus* were wrongly diagnosed as carrying methicillin-susceptible *S. aureus* rather than MRSA, given the low sequence conservation between *mecC* and *mecA*. The importance of Holmes' work was highlighted by its dissemination (prior to full publication) via the EuroStaph network (an internet forum used by European clinical microbiology labs), which led to the immediate adoption of the Holmes group's new *mecC*-specific PCR primers, or use of the group's whole genome sequence data, for MRSA

mecC detection by MRSA reference laboratories in England & Wales, Scotland, Denmark, the Netherlands, France, Germany, Sweden, Norway and Belgium. Subsequently the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines for MRSA testing have been amended to include *mecC* MRSA [corroboration source 10]

In April 2011, a meeting was called by Prof Maria Zambon, Director of the Health Protection Agency (HPA, now called Health Protection England) Microbiology section at Colindale, to explore the potential impact of the discovery of *mecC* on human health and MRSA screening programmes, and to discuss the epidemiology of the new MRSA in humans and animals. The outcomes of the risk assessments undertaken at this meeting have since been used to inform the Department of Health, Public Health England and other relevant bodies about the potential risks of new MRSA strains, and how they might be mitigated [corroboration sources 7 & 8]. A recent publication providing evidence of animal-to-human transmission has been cited in intra-governmental correspondence in the United States [corroboration source 9].

Based on the work of the Holmes group, and in collaboration with them, UK Department of Health and international microbiology reference laboratories have now developed a number of further tests to distinguish *mecC* from *mecA* that are more appropriate for clinical laboratory use (i.e. multiplex PCRs that include species confirmation and *mecA*, in addition to detection of *mecC*) for accurate diagnosis of MRSA in infected patients [references 2,3,5 above]. For example, Reference 2 (section 3) includes the statement "The real-time quadruplex PCR was introduced into the HPA-SRU in August 2011, replacing the McDonald et al. real-time triplex PCR (nuc, lukS-PV and *mecA*)". As a direct result of Holmes' research, the PCR tests used to investigate MRSA isolates in Health Protection England (formerly the HPA) *Staphylococcus* Reference Unit, the Scottish MRSA Reference Laboratory, the *Staphylococcus* Laboratory at the Statens Serum Institut in Denmark, and other international MRSA reference laboratories have been changed to enable detection of *mecC* MRSA [corroborating source 2].

In hospital settings, there is increasing use of automated PCR testing, and test equipment is currently being updated to make provision for *mecC* screening of patients presenting with suspected MRSA, by the main test providers (Roche and Becton Dickinson). A commercial microarray-based MRSA test system incorporating *mecC* sequence data has been developed and is now being marketed (Greiner bio-one, Genspeed MRSA Test System) [corroboration source 5]. A commercial qRT-PCR system incorporating primers for *mecC* is marketed by ELITech (MRSA/SA ELITe MGB® Kit) [corroboration source 6]. The European Union Reference Laboratory for Antibiotic Resistance has published a recommended PCR protocol for the detection of MRSA incorporating primers for *mecC* [corroboration source 4].

On publication of the original discovery [1] considerable media interest was generated across the world. There were reports in The Times, Telegraph, Independent, Guardian [corroborating source 1] and New Scientist in the UK. International newspaper coverage included the Los Angeles Times and El Pais. The story was also covered by interviews with Dr Holmes aired on the BBC Radio 4 Today programme, CBS television in the USA, and an extended interview on the BBC 1 programme Countryfile.

The discovery of this novel MRSA has been discussed at numerous industry and governmental committees including the Defra antimicrobial resistance co-ordination group [corroborating source 7] and the Advisory Committee on Antimicrobial Resistance & Healthcare-Associated Infection [corroborating source 8]. European MRSA recommended testing protocols and methodologies have been changed as a result of this research [corroborating sources 4 and 10].

Publication of evidence of zoonotic transmission of *mecC* MRSA was reported in the Independent and the Daily Mail. This work was also cited in a letter from a US congresswoman to the Commissioner of the Food and Drug Administration [corroborating source 9].

5. Sources to corroborate the impact (indicative maximum of 10 references)

1. International media interest was generated by the original publication of this discovery in June 2011 (reference 1 above). One example of the media coverage to corroborate this is The Guardian (June 3rd 2011) – “New strain of MRSA superbug may have spread from cattle to humans” <http://www.theguardian.com/science/2011/jun/03/new-strain-mrsa-cattle-humans>
2. The Stegger and Pichon references [references 2 & 3 above] indicate how the national Staphylococcal reference laboratories in England (HPE Colindale) and Denmark have developed new testing regimes now used for all their MRSA testing to incorporate sequence data generated by this research. The first author and the senior author are staff from the reference laboratories and were responsible for drafting the paper. Holmes is included as an author to acknowledge his discovery of the gene and contribution to the design of the assay. Reference 2 includes the statement "The real-time quadruplex PCR was introduced into the HPA-SRU in August 2011, replacing the McDonald et al. real-time triplex PCR (nuc, lukS-PV and mecA)".
3. Readers of the Lancet Infectious Diseases voted that the paper that first described the discovery (Section3, Ref 1) was the 4th most influential paper published by the journal over the last 10 years. This is a high impact clinical journal that reflects the influence of this discovery beyond the immediate scientific or academic interest. McConnell (2011) Readers' ten most influential. Lancet Infect Dis, 11, 726-727.
<http://www.sciencedirect.com/science/article/pii/S1473309911702420#>
4. Protocol for PCR amplification of *mecA*, *mecC* (*mecA*_{LG251}), *spa* And PVL recommended by the EU Reference Laboratory for antimicrobial resistance (EURL-AR). 2nd Version, September 2012. http://www.crl-ar.eu/data/images/protocols/pcr_spa_pvl_meca_mecc_sept12.pdf
5. Genspeed MRSA Test System (marketing brochure) 2012
http://www.gbo.com/documents/980286_GENSPEED_MRSA_e_rev01_0812_small.pdf
6. Elitech press releases; July 2nd, 2012 on Real-time PCR MRSA kit that detects the *mecA*_{LG251} gene and August 27th 2012 on nomenclature recommendation for the unique methicillin-resistant *S. aureus* variant *mecA*_{LG251}
7. Defra antimicrobial resistance co-ordination group (DARC) minutes for 2 August 2011 & 22 May 2012
8. Advisory Committee on Antimicrobial Resistance & Healthcare-Associated Infection (ARHAI) minutes for 22 June 2011
9. Letter from Congresswoman to Commissioner of the Food and Drug Administration, USA (26 March 2013) concerning the implications of publication of reference 9.
10. European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance (December 2012)
http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Consultation/EUCAST_guidelines_detection_of_resistance_mechanisms_121222.pdf