Ethical approval

The current study was approved by the Research Ethics Committee of Hospital Clínico Universitario INCLIVA (September, 2019).

Conflict of interest

The authors have no conflict of interest.

Acknowledgments

We are grateful to residents and staff at the Microbiology Service of Hospital Clínico Universitario. Ignacio Torres holds a contract (Río Hortega Contract; CM20/00090) funded by the Carlos III Health Institute (co-financed by the European Regional Development Fund, ERDF/FEDER).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.eimce.2023.01.009.

References

1. Ibrahim EH, Sherman G, Ward S, Fraser VJ, Kollef MH. The influence of inadequate antimicrobial treatment of bloodstream infections on patient outcomes in the ICU setting. Chest. 2020;118:146–55.
2. Kang CI, Kim SH, Park WB, Lee KD, Kim HB, Kim EC, et al. Bloodstream infections caused by antibiotic-resistant Gram-negative bacilli: risk factors for mortality and impact of inappropriate initial antimicrobial therapy on outcome. Antimicrob Agents Chemother. 2005;49:760–6.
3. Schuts EC, Hulscher MEJL, Mouton JW, Verduin CM, Cohen-Stuart JWT, Overdiek HWPM, et al. Current evidence on hospital antimicrobial stewardship objectives: a systematic review and meta-analysis. Lancet Infect Dis. 2016;16:847–56.
4. Shorr AF, Micek ST, Welch EC, Doherty JA, Reichley RM, Kollef MH. Inappropriate antibiotic therapy in Gram-negative sepsis increases hospital length of stay. Crit Care Med. 2011;39:46–51.
5. Oviaño M, Bou G. Matrix-assisted laser desorption ionization-time of flight mass spectrometry for the rapid detection of antimicrobial resistance mechanisms and beyond. Clin Microbiol Rev. 2018;32, e00037–18.
6. Torres I, Albert E, Giménez E, Olea B, Valdivia A, Pascual T, et al. Performance of a MALDI-TOF mass spectrometry-based method for rapid detection of third-generation oxymino-cephalosporin-resistant *Escherichia coli* and *Klebsiella* spp. from blood cultures. Eur J Clin Microbiol Infect Dis. 2021;40:1925–32.
7. https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_10.0_Breakpoint_Tables.pdf.
8. Torres Fink I, Tormo Palop N, Borrás Salvador R, Buesa Gómez J, Gimeno Cardona C, Navarro Ortega D. Evaluation of the DNA microarray "AMR Direct Flow Chip Kit" for detection of antimicrobial resistance genes from Gram-positive and Gram-negative bacterial isolated colonies. Enferm Infecc Microbiol Clin (Engl Ed). 2019;37:454–7.
9. Sánchez-Carrillo C, Pescador P, Ricote R, Fuentes J, Losada C, Candela A, et al. Evaluation of the Alfred AST® system for rapid antimicrobial susceptibility testing directly from positive blood cultures. Eur J Clin Microbiol Infect Dis. 2019;38:1665–70.

David Sánchez [a], Javier Colomina [a], David Navarro [a,b,*], Ignacio Torres [a]

[a] *Microbiology Service, Hospital Clínico Universitario, Instituto de Investigación INCLIVA, Valencia, Spain*
[b] *Department of Microbiology, School of Medicine, University of Valencia, Spain*

* Corresponding author.
E-mail address: david.navarro@uv.es (D. Navarro).

https://doi.org/10.1016/j.eimce.2023.01.009

Methicillin-resistant *Staphylococcus aureus* bacteremia carrying the *mecC* gene

Bacteriemia por Staphylococcus aureus *resistente a meticilina portador del gen* mecC

In methicillin-resistant *Staphylococcus aureus (S. aureus)* (MRSA), resistance to β-lactams is due to alteration of penicillin-binding proteins (PBPs). This resistance mechanism arises from the acquisition of the *mecA* gene, encoding a PBP2a with low affinity for β-lactams.[1,2] In 2011, a new resistance gene was described, the *mecC* gene that encodes PBP2c. Its presence has spread[3] and for this reason its identification is essential to establish adequate antibiotic therapy.

We present the case of a patient with *mecC* MRSA bacteraemia.

This was a 70-year-old man with multiple pathologies, who attended the accident and emergency department due to poor pain control after a fall. He was diagnosed with dorsal fractures and presented with hyperbilirubinaemia with jaundice, and it was decided to admit him. His condition worsened and blood and urine cultures were taken. Empirical treatment with piperacillin-tazobactam was started and he was admitted to the Intensive Care Unit (ICU) with suspicion of septic shock.

In the Gram stain of the blood culture, Gram-positive cocci were observed in clusters that were identified by PCR (Xpert® MRSA/SA BC, Cepheid) as methicillin-susceptible *S. aureus* (MSSA), so cloxacillin was added. After performing culture and antibiogram following the EUCAST breakpoints,[4] resistance to β-lactams was observed. Immunochromatography was performed (CLEARVIEW™ PBP2a SA Culture Colony Test, Abbott) with a negative result for PBP2a. With suspicion of infection by *mecC* MRSA, a second commercial PCR was performed (FilmArray® Blood Culture Identification Panel, bioMérieux) with a positive result for the targets *mecA/C* and MREJ. Furthermore, by microdilution (MicroScan Pos Combo Panel Type 33, Beckman Coulter), MIC values for oxacillin and cefoxitin were 2 mg/l and >4 mg/l, respectively, and the rest of the families of antibiotics were interpreted as susceptible. The presence of the *mecC* gene was confirmed by ẗome PCRẅith specific molecular targets and sequencing. Antibiotic therapy was changed to daptomycin and levofloxacin but the patient died 14 days after admission.

Recent publications suggest that the appearance of *mecC* MRSA predates the use of antibiotics,[5] and that transmission to humans can occur through contact with animals.[3,6,7] In addition, colonisation, advanced age and underlying disease have also been associated with infection.[8] In our case, the patient confirmed contact with livestock and in the nasal sample for the study of resistant bacteria collected on his admission to the ICU, *mecC* MRSA was also isolated, so the patient was colonised and the infection developed.

In microbiological diagnosis, the discovery of the *mecC* gene with 70% nucleotide homology to the *mecA*gene, and PBP2c encoding with 62% amino acid sequence similarity to PBP2a means tests targeting the detection of the *mecA* gene and PBP2a misidentify a *mecC* MRSA isolate as MSSA.[2,9] Therefore, performing an antibiogram and using specific techniques is essential.[2] *mecC* MRSA accounts for less than 1% of all MRSA, and the majority

DOI of refers to article: https://doi.org/10.1016/j.eimc.2023.01.008

belong to the clonal complex (CC) CC130. In Spain, within this CC, the most common sequence type and *spa* type are ST1945 and t843, respectively.[5,8] In our patient, the initial PCR and the immunochromatographic technique that detects the presence of PBP2a identified the isolate as MSSA. However, after carrying out the antibiogram, resistance was confirmed by specific PCR and sequencing, also demonstrating the presence of the *mecC* gene integrated into the *SCCmec* XI cassette chromosome. Furthermore, through multilocus sequence typing (MLST), it was catalogued within CC130, and through molecular typing by PCR and subsequent sequencing of the *spa* type, a new *spa* type registered as t20888, within ST1945, was detected.

In conclusion, this was a case of invasive infection by *mecC* MRSA with a new *spa* type and with a fatal outcome in which the patient met the risk factors for infection by this microorganism: close contact with livestock, underlying disease, advanced age and nasal colonisation. In microbiology laboratories, it is important to carry out a manual antibiogram along with the use of rapid molecular techniques, since mechanisms not included in the molecular targets of commercial PCRs may be identified. This is essential for choosing the most appropriate antibiotic therapy.

Acknowledgements

We would like to thank Carmen Torres and her working group from the Universidad de La Rioja [University of La Rioja] for helping to characterise the strain of *S. aureus* carrying the *mecC* gene.

References

1. Lakhundi S, Zhang K. Merhicillin-resistant *Staphylococcus aureus*: molecular characterization, evolution, and epidemiology. Clin Microbiol Rev. 2018;31, http://dx.doi.org/10.1128/CMR.00020-18, e00020-18.
2. García-Álvarez L, Holden MTG, Lindsay H, Webb CR, Brown DFJ, Curran MD, et al. Meticillin-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, http://dx.doi.org/10.1016/S1473-3099(11)70126-8.
3. García-Garrote F, Cercenado E, Marín M, Bal M, Trincado P, Corredoira J, 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, http://dx.doi.org/10.1093/jac/dkt327.
4. The European Committee on Antimicrobial Susceptibility Testing. Clinical breakpoints-bacteria version 11.0.2022. [accessed 2 Nov 2022]. Available from: www.eucast.org.
5. Larsen J, Raisen CL, Ba X, Sadgrove NJ, Padilla-Gonzalez GF, Simmonds MSJ, et al. Emergence of methicillin resistance predates the clinical use of antibiotics. Nature. 2022;602:135–41, http://dx.doi.org/10.1038/s41586-021-04265-w.
6. Harrison EM, Paterson GK, Holden MTG, Larsen J, Stegger M, Larson AR, et al. Whole genome sequencing identifies zoonotic trasmission of MRSA isolates with the novel *mecA* homologue *mecC*. EMBO Mol Med. 2013;5:504–15, http://dx.doi.org/10.1002/emmm.201202413.
7. Angen O, Stegger M, Larsen J, Lilje B, Kaya H, Pedersen KS, et al. Report of *mecC*-carrying MRSA in domestic swine. J Antimicrob Chemother. 2017;72:60–3, http://dx.doi.org/10.1093/jac/dkw389.
8. Lozano C, Fernández-Fernández R, Ruiz-Ripa L, Gómez P, Zarazaga M, Torres C. Human *mecC*-carrying MRSA: clinical implications and risk factors. Microorganisms. 2020;8:1615, http://dx.doi.org/10.3390/microorganisms8101615.
9. Ba X, Harrison EM, Lovering AL, Gleadall N, Zadiks R, Parkhill J, et al. Old drug to treat resistan bugs: methicilin-resistant *Staphylococcus aureus* isolates with *mecC* are susceptible to a combination of penicillin and clavulanic acid. Antimicrob Agents Chemother. 2015;59:7396–404, http://dx.doi.org/10.1128/AAC.01469-15.

Irati Arregui Garcia [a,*], M. Eugenia Portillo [a,b], Luis Torroba Álvarez [a,b], Carmen Ezpeleta Baquedano [a,b]

[a] *Servicio de Microbiología Clínica, Hospital Universitario de Navarra, Pamplona, Spain*
[b] *Instituto de Investigación Sanitaria de Navarra (IdisNA), Pamplona, Spain*

* Corresponding author.
E-mail address: iratiarreguig@gmail.com (I. Arregui Garcia).

https://doi.org/10.1016/j.eimc.2023.04.016

High frequency of *Streptococcus pneumoniae* serotype 3 in negative pleural fluid cultures from paediatric samples obtained in the Madrid region from 2018 to 2022, detected by direct identification using PCR-reverse-hybridization strip-based assay

Elevada frecuencia del serotipo 3 de Streptococcus pneumoniae *detectado mediante una técnica de PCR e hibridación reversa, en muestras pediátricas de líquido pleural con cultivo negativo, obtenidas en la Comunidad de Madrid entre 2018 y 2022*

The first pneumococcal conjugate vaccine introduced in 2006 in the paediatric immunization calendar of the Madrid region was the 7-valent (PCV7), which was substituted in 2010 by the 13-valent pneumococcal conjugate vaccine (PCV13). In 2012, PCV13 was withdrawn from the Madrid paediatric public immunization programme, being prescribed privately. In 2015, the PCV13 was re-introduced at the Spanish national paediatric public vaccine scheme.[1] The Quellung reaction is the gold standard for pneumococcal serotyping. This method requires strains isolated in culture. However, in samples as parapneumonic effusion (PPE) and pleural empyema (PE), commonly grouped as PPE/PE,[2] the bacterial growing is fastidious and the availability of colonies may be difficult. The objective of this work was to identify the not growing *Streptococcus pneumoniae* serotypes (SPNGST) causing pleural infection in children, in which the standard microbiological culture shows negative results.

Thirty-five PFS from paediatric patients (aged six months to 8 years; mean 3.4 years, standard deviation 2.1) with negative culture results, obtained between September 2018 and December 2022, were processed for the detection of the α-fucosidase gene using a real time PCR method (*Streptococcus pneumoniae* alpha-fucosidase gene Genesig® Advanced Kit; Primerdesign Ltd, United Kingdom). Positive α-fucosidase samples were subsequently tested by a PCR reverse-hybridization strip-based assay (S. PneumoStrip test; Operon S.A., Zaragoza, Spain) that allows serotype identification.

The α-fucosidase real-time PCR method was positive in 30 of the 35 samples studied (85.7%). The PCR reverse-hybridization assay showed positive results for the *lytA*, *cpsA* and *ply* genes in 28 of the 30 samples (93.3%). The serotypes detected by this technique in these 28 samples were twenty-one serotype 3 (75%), two serotype 8 (7.1%), one serotype 19A (3.6%), one serotype 1 (3.6%), one serotype 10A (3.6%), one 9A/9V (3.6%) and one 33F/33A (3.6%). Table 1 shows the distribution of PCR SPNGST detected in PFS according to time. Most serotype 3 cases occurred in 2022 (17/21). The only one PCV13 covered serotype 19A was identified in 2018. The only one PCV13 serotype 1 case was detected in 2020. The number of serotype 3 cases detected in PFS after 2020 was significantly

DOI of refers to article: https://doi.org/10.1016/j.eimc.2023.02.001