

Absence Of Methicillin-Resistant *Staphylococcus aureus* (MRSA) In Cattle From Portugal: A One Health Approach

This article was published in the following Dove Press journal:
Infection and Drug Resistance

Susana Correia¹⁻⁴
Vanessa Silva¹⁻⁴
Juan García-Díez⁵
Paula Teixeira⁶
Kevin Pimenta¹⁻⁴
María Teresa Tejedor-Junco⁷
Soraia Oliveira¹⁻⁴
Gilberto Igrejas²⁻⁴
Patricia Poeta^{1,4}

¹Microbiology and Antibiotic Resistance Team (MicroART), Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal; ²Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal; ³Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal; ⁴LAQV-REQUIMTE, Faculty of Science and Technology, University Nova of Lisbon (FCT-UNL), Lisbon, Portugal; ⁵Animal and Veterinary Research Centre (CECAV), Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal; ⁶Associação de Criadores do Maronês (ACM), Cooperativa Agrícola de Vila Real, Vila Real, Portugal; ⁷Research Institute of Biomedical and Health Sciences, University of Las Palmas de Gran Canaria, Canary Islands, Spain

Correspondence: Patricia Poeta
Microbiology and Antibiotic Resistance Team (MicroART), Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real 5000-801, Portugal
Tel +351 259 350466
Fax +351 259 350629
Email ppoeta@utad.pt

Antimicrobial resistance (AMR) is acknowledged today as one of the most concerning threats to global human health since the future and confines of modern medicine continue to be defined and shaped by the success of antimicrobial therapy.¹ As resistant bacteria may spread without recognizing human-animal or geographic borders, many major international organizations have stated that the rising threat of AMR must be addressed through a concerted and multisectoral One Health approach involving humans, animals and the environment. *Staphylococcus aureus* is a hardy, adaptable, opportunistic pathogenic agent that is able to thrive in diverse environments.²⁻⁴ It is often present in the natural flora of the human and animal nose and skin, being also recovered from food, food production systems and the environment, causing a range of illnesses from minor skin infections and food poisoning to life-threatening diseases.^{2,3,5} Methicillin-resistant *Staphylococcus aureus* (MRSA) represents a global pandemic threat being resistant to most beta-lactam antibiotics and to other major antimicrobial drug classes.^{2,3} MRSA was first established in the healthcare setting, emerging afterwards in the community, animals and in food of animal origin, disclosing novel MRSA reservoirs.^{4,5} Presently, *S. aureus* represents a leading cause of livestock infection, resulting in great economic losses in the food industry.^{4,6} Livestock-associated MRSA (LA-MRSA) has also been reported among human patients who have been in contact with infected or colonized animals, being the causing agent of sepsis, endocarditis, osteomyelitis, pneumonia and other major invasive infections.^{3,5,7} MRSA in animals was first reported in a case of bovine mastitis in the early 1970s and since then MRSA has been progressively identified in farm animals and in humans contacting with these animals, revealing MRSA as a major veterinary and zoonotic pathogen.^{4,7} In order to follow a One Health approach to determine the prevalence and transmission of MRSA in cattle, mouth and nose swabs of 49 healthy cows (11 Friesians and 28 cross-breeds) were recovered together with 19 human, 13 water and 20 soil samples from the animals' handlers and environments (Figure 1). Sampling was performed from February to April 2019 in 9 different locations within a radius of approximately four kilometers from the CHTMAD hospital center in Vila Real, Portugal (Vila Marim, Sirarelhos, Lordelo, Sapiões, Guilpilhares, Quintelas, Relva, Borbela and Agarez; Figure 2). Mouth and nose swabs were obtained from all consenting handlers in regular contact with the sampled animals; water and soil samples were

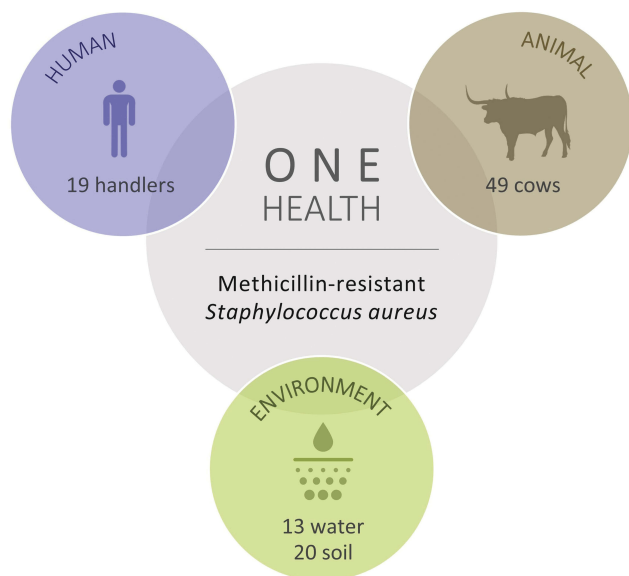


Figure 1 Total number of cattle, human, water and soil samples screened for methicillin-resistant *Staphylococcus aureus* (MRSA).



Figure 2 Satellite 3D image of the 9 different sampling locations in relation to the CHTMAD hospital centre in Vila Real, Portugal.

collected from different points of each corresponding drinking trough and cowshed, respectively. Swabs were collected into Stuart transport media; water was recovered into 500 mL PET flasks with 60 mg/L sodium thiosulphate and soil was gathered into appropriate zipper seal sample bags. Samples were processed in the same day or stored at 4°C for a maximum of 24 h. Swabs and soil samples were incubated into Brain Heart Infusion broth with 6.5% (w/v) NaCl for 48 h at 37°C and after seeded on Oxacillin Resistance Screening Agar Base (ORSAB) supplemented with 2 mg/L oxacillin. Water samples were filtered through 47 mm 0.2 µm filters that were further placed on ORSAB plates with 2 mg/L oxacillin. All plates were incubated for 24 to 48 h at 37°C and after screened for presumptive

MRSA colonies. Livestock and livestock production systems are known to be potential reservoirs for MRSA clones with the capacity to cross the species barrier, endure host-adaptive evolution, and become established in human populations worldwide as successful epidemic lineages.^{3,6} Hence, it was expected that MRSA strains could be recovered among the animal, human and environmental samples screened, that would allow the study of the occurrence and transmission of different MRSA lineages and their resistance determinants. However, no presumptive MRSA colonies were recovered from the total of 101 samples screened in selective ORSAB media. Since cattle and their respective handlers and environments are ordinarily colonized with MRSA,^{4,7} the observed absence of this major human and animal pathogen represents a serendipitous result, with a very positive impact in food safety and public health. Although the proximity to the main hospital center of the region, samples were collected from extensive production systems in higher mountain rural areas. These systems mainly use natural resources and do not regularly use antimicrobials in subtherapeutic doses, which would result in lower levels of antibiotic pressure selecting for MRSA. Through a One Health approach, this study revealed that the screened Friesian and crossbreed cattle, their handlers and their surrounding environments do not represent reservoirs for MRSA, revealing that the studied cattle from Vila Real, Portugal are, in this aspect, safe from the zoonotic point of view. Nevertheless, studying the prevalence and transmission of methicillin-susceptible *S. aureus* harboring resistance determinants for other important antimicrobial drugs would be interesting as well as the extension of the study to other bacterial species that also represent major global AMR threats.

Ethical Statements

This work was approved Institutionally by University of Trás-os-Montes and Alto Douro Board and written informed consent was obtained from all subjects.

Acknowledgments

This work was funded by the R&D Project CAREBIO2 - Comparative assessment of antimicrobial resistance in environmental biofilms through proteomics - towards innovative theranostic biomarkers, with reference NORTE-01-0145-FEDER-030101 and PTDC/SAU-INF/30101/2017, financed by the European Regional Development Fund (ERDF) through the Northern Regional Operational Program (NORTE 2020)

and the Foundation for Science and Technology (FCT). This work was supported by the Associate Laboratory for Green Chemistry - LAQV which is financed by national funds from FCT/MCTES (UID/QUI/50006/2019). Vanessa Silva is supported by national funds through FCT/MCTES and by the European Social Fund through POCH/FSE under the PhD grant SFRH/BD/137947/2018.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Ahmad M, Khan AU. Global economic impact of antibiotic resistance: a review. *J Glob Antimicrob Resist*. 2019. doi:10.1016/j.jgar.2019.05.024
2. Gajdács M. The continuing threat of methicillin-resistant *Staphylococcus aureus*. *Antibiotics*. 2019;8(2):52. doi:10.3390/antibiotics8020052
3. Dweba CC, Zishiri O, El Zowalaty M. Methicillin-resistant *Staphylococcus aureus*: livestock-associated, antimicrobial, and heavy metal resistance. *Infect Drug Resist*. 2018;11:2497–2509. doi:10.2147/IDR.S175967
4. Aires-de-Sousa M. Methicillin-resistant *Staphylococcus aureus* among animals: current overview. *Clin Microbiol Infect*. 2017;23(6):373–380. doi:10.1016/j.cmi.2016.11.002
5. Boswihi SS, Udo EE. Methicillin-resistant *Staphylococcus aureus*: an update on the epidemiology, treatment options and infection control. *Curr Med Res Pract*. 2018;8(1):18–24. doi:10.1016/j.cmrp.2018.01.001
6. Spoor LE, McAdam PR, Weinert LA, et al. Livestock origin for a human pandemic clone of community-associated methicillin-resistant *Staphylococcus aureus*. Baquero F, ed. *MBio*. 2013;4(4):e00356–13. doi:10.1128/mBio.00356-13
7. Goerge T, Lorenz MB, van Alen S, Hübner N-O, Becker K, Köck R. MRSA colonization and infection among persons with occupational livestock exposure in Europe: prevalence, preventive options and evidence. *Vet Microbiol*. 2017;200:6–12. doi:10.1016/j.vetmic.2015.10.027

Infection and Drug Resistance

Dovepress

Publish your work in this journal

Infection and Drug Resistance is an international, peer-reviewed open-access journal that focuses on the optimal treatment of infection (bacterial, fungal and viral) and the development and institution of preventive strategies to minimize the development and spread of resistance. The journal is specifically concerned with the epidemiology of

antibiotic resistance and the mechanisms of resistance development and diffusion in both hospitals and the community. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/infection-and-drug-resistance-journal>