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Letter to the Editor

## First report of linezolid-resistant *cfr*-positive methicillin-resistant *Staphylococcus aureus* in humans in Portugal

*Sir,*

Linezolid and vancomycin are the most reliable therapeutic agents to treat complicated soft tissue infections caused by multidrug-resistant methicillin-resistant *Staphylococcus aureus* (MRSA) strains. However, despite the fact that the prevalence of linezolid-resistant MRSA strains (LR-MRSA) remains overall low, in the past few years resistance to linezolid has been reported among human patients worldwide, imposing a public-health concern, in particular when associated with a mobile gene such as *cfr* [1]. The aim of this study was to identify LR-MRSA isolates recovered from infected diabetic foot ulcers and to characterise their antimicrobial resistance profiles and genetic lineage.

Ulcer samples were collected from type 2 diabetic inpatients ($n$ = 45) with infected foot ulcers. Each ulcer specimen for culture was obtained within 48 h of hospital admission by scraping the ulcer base. Isolates were seeded onto Oxacillin Resistance Screening Agar Base with 2 mg/L oxacillin for isolation of MRSA strains. The minimum inhibitory concentration (MIC) of linezolid was determined using Etest strips (Liofilchem®). Susceptibility to 14 other antimicrobials was determined by the Kirby–Bauer disk diffusion method and was interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2018 guidelines, with the exception of kanamycin which followed Clinical and Laboratory Standards Institute (CLSI) standards. The presence of antimicrobial resistance genes was studied by PCR and sequencing (Supplementary Table S1). All isolates were characterised by accessory gene regulator (*agr*) typing, staphylococcal protein A (*spa*) typing, staphylococcal cassette chromosome *mec* (SCC*mec*) typing and multilocus sequence typing (MLST).

From the 45 samples, 28 (66%) MRSA isolates were found, of which 3 (from three different patients) showed phenotypic resistance to linezolid with MICs ranging from 8 to 16 mg/L. Resistance to linezolid may be mediated by the *cfr* gene, which affects the binding site of the drug by 23S rRNA methylation; and/or by mutations in one or more alleles of the 23S rRNA, which decreases the affinity of ribosomes for linezolid [2]. The present study investigated the occurrence of the *cfr* gene, which was found to be carried by all three LR-MRSA isolates (Table 1). Besides conferring resistance to linezolid, this gene is also responsible for combined resistance to several groups of antibiotics such as lincosamides, group A streptogramins and phenicols [3]. *cfr*-positive clinical MRSA strains have been reported in other countries both in humans and animals; however, to the best of our knowledge, this gene has only been detected in one dog with severe bilateral otitis in Portugal [4]. Phenotypic resistance to tetracycline ($n$ = 2), ciprofloxacin ($n$ = 2), erythromycin ($n$ = 2), clindamycin ($n$ = 2), fusidic acid ($n$ = 2), gentamicin ($n$ = 1) and trimethoprim/sulfamethoxazole (SXT) ($n$ = 2) was also found in the LR-MRSA isolates. Both isolates showing resistance to tetracycline harboured the *tetL* and *tetO* genes, and one also harboured the *tetK* gene. The *dfrA* and *dfrK* genes conferring resistance to SXT were detected in both SXT-resistant isolates, whilst the *dfrG* gene was found in only one isolate. One LR-MRSA strain was ST22, SCC*mec* type IV (ST22-MRSA-IV), *spa* type t747 and *agr* type II. ST22-MRSA-IV, also known as epidemic MRSA-15 (EMRSA-15), is a pandemic clone reported to be a frequent nosocomial clone detected in MRSA strains that have the ability to adapt to the introduction of new and different antimicrobials into the hospital environment. This clone is the most frequently found in Portuguese hospitals, in particular in hospitals located in the north of Portugal [5]. The second LR-MRSA isolate was assigned to ST105 (CC5), SCC*mec* II, *spa* type t002 and *agr* type II. The ST105-MRSA-II/t002 clone, which differs from ST5 by one point mutation in the *yqiL* locus, was previously reported in Portuguese hospitals, nevertheless the MRSA isolates did not show resistance to linezolid [5]. Furthermore, clone ST105-MRSA-II/t002 was also found in other countries in clinical MRSA strains but lacking the *cfr* gene or resistance to linezolid. The last LR-MRSA isolate belonged to ST8 (CC8), SCC*mec* IV, *spa* type t1476

**Table 1**
Characteristics of the linezolid-resistant methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated from infected diabetic foot ulcers in Portugal.

| Isolate | Methicillin resistance gene | Resistance | | Linezolid MIC (mg/L) | Typing | | | |
|---|---|---|---|---|---|---|---|---|
| | | Phenotype | Genotype | | ST | *spa* | SCC*mec* | *agr* |
| VS2702 | *mecA* | PEN/FOX/LNZ | *blaZ*, *cfr* | 8 | 22 | t747 | IV | II |
| VS2703 | *mecA* | PEN/FOX/TET/LNZ/SXT/CIP/ERY/CLI/FUS | *blaZ*, *cfr*, *ermA*, *tetO*, *tetL*, *mph*(C), *cfr*, *dfrA*, *dfrK* | 16 | 105 | t002 | II | II |
| VS2704 | *mecA* | PEN/FOX/TET/LNZ/SXT/CIP/ERY/CLI/FUS/GEN | *blaZ*, *cfr*, *aac(6′)-Ie–aph(2″)-Ia*, *tetK*, *tetO*, *tetL*, *cfr*, *dfrA*, *dfrG*, *dfrK* | 8 | 8 | t1476 | IV | I |

MIC, minimum inhibitory concentration; ST, sequence type; *spa*, staphylococcal protein A; SCC*mec*, staphylococcal cassette chromosome *mec*; *agr*, accessory gene regulator; PEN, penicillin; FOX, cefoxitin; LNZ, linezolid; TET, tetracycline; SXT, trimethoprim/sulfamethoxazole; CIP, ciprofloxacin; ERY, erythromycin; CLI, clindamycin; FUS, fusidic acid; GEN, gentamicin.

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and *agr* type I. ST8-MRSA-IV clone, also known as USA300, is a community-acquired MRSA clone that has been imported from the community to the hospital and is considered pandemic and an emerging nosocomial clone as it has been isolated repeatedly from every continent and is one of the five globally predominant community-acquired MRSA [6]. Besides, this clone is recognised as a cause of skin and soft-tissue infection. Other studies have reported the presence of the *cfr* gene in USA300 clone [7,8]. The detection of three clinical MRSA strains carrying the *cfr* gene encoding resistance to linezolid is alarming since this gene had not been yet found circulating among the human *S. aureus* population in Portugal. Besides, in this study, linezolid-resistant strains were associated with the pandemic clones EMRSA-15, USA300 and New York/Japan (related), which is a cause for enormous concern.

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## Competing interests

None declared.

## Ethical approval

Not required.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jgar.2019.05.017.

## References

[1] Antonelli A, D'Andrea MM, Galano A, Borchi B, Brenciani A, Vaggelli G, et al. Linezolid-resistant *cfr*-positive MRSA, Italy. J Antimicrob Chemother 2016;71:2349–51, doi:http://dx.doi.org/10.1093/jac/dkw108.

[2] Dong W, Chochua S, McGee L, Jackson D, Klugman KP, Vidal JE. Mutations within the *rplD* gene of linezolid-nonsusceptible *Streptococcus pneumoniae* strains isolated in the United States. Antimicrob Agents Chemother 2014;58:2459–62, doi:http://dx.doi.org/10.1128/AAC.02630-13.

[3] Long KS, Poehlsgaard J, Kehrenberg C, Schwarz S, Vester B. The Cfr rRNA methyltransferase confers resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A antibiotics. Antimicrob Agents Chemother 2006;50:2500–5, doi:http://dx.doi.org/10.1128/AAC.00131-06.

[4] Seixas R, Monteiro V, Carneiro C, Vilela CL, Oliveira M. First report of a linezolid-resistant MRSA isolated from a dog with a severe bilateral otitis in Portugal. Rev Vet 2011;22:81–4.

[5] Espadinha D, Faria NA, Miragaia M, Lito LM, Melo-Cristino J, de Lencastre H, et al. Extensive dissemination of methicillin-resistant *Staphylococcus aureus* (MRSA) between the hospital and the community in a country with a high prevalence of nosocomial MRSA. PLoS One 2013;8:e59960.

[6] Mediavilla JR, Chen L, Mathema B, Kreiswirth BN. Global epidemiology of community-associated methicillin resistant *Staphylococcus aureus* (CA-MRSA). Curr Opin Microbiol 2012;15:588–95, doi:http://dx.doi.org/10.1016/j.mib.2012.08.003.

[7] Locke JB, Zuill DE, Scharn CR, Deane J, Sahm DF, Goering RV, et al. Identification and characterization of linezolid-resistant *cfr*-positive *Staphylococcus aureus* USA300 isolates from a New York City medical center. Antimicrob Agents Chemother 2014;58:6949–52, doi:http://dx.doi.org/10.1128/AAC.03380-14.

[8] Shore AC, Brennan OM, Ehricht R, Monecke S, Schwarz S, Slickers P, et al. Identification and characterization of the multidrug resistance gene *cfr* in a Panton–Valentine leukocidin-positive sequence type 8 methicillin-resistant *Staphylococcus aureus* IVa (USA300) isolate. Antimicrob Agents Chemother 2010;54:4978–84, doi:http://dx.doi.org/10.1128/AAC.01113-10.

Vanessa Silva[a,b,c,d]
[a]*MicroART - Microbiology and Antibiotic Resistance Team, Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal*
[b]*Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal*
[c]*Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal*
[d]*Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisboa, Lisboa, Caparica, Portugal*

Francisco Almeida
*Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal*

Adriana Silva[a,b,c]
[a]*MicroART - Microbiology and Antibiotic Resistance Team, Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal*
[b]*Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal*
[c]*Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal*

Susana Correia[a,b,c,d]
[a]*MicroART - Microbiology and Antibiotic Resistance Team, Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal*
[b]*Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal*
[c]*Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal*
[d]*Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisboa, Lisboa, Caparica, Portugal*

José António Carvalho
Ana Paula Castro
*Centro Hospitalar de Trás-os-Montes e Alto Douro E.P.E., Vila Real, Portugal*

Eugénia Ferreira[a,b]
Vera Manageiro[a,b]
Manuela Caniça[a,b]
[a]*National Reference Laboratory of Antibiotic Resistances and Healthcare Associated Infections (NRL-AMR/HAI), Department of Infectious Diseases, National Institute of Health Dr Ricardo Jorge, Av. Padre Cruz, 1649-016, Lisbon, Portugal*
[b]*Centre for the Studies of Animal Science, Institute of Agrarian and Agri-Food Sciences and Technologies, Oporto University, Oporto, Portugal*

Gilberto Igrejas[a,b,c]
[a]*Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal*
[b]*Functional Genomics and Proteomics Unit, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal*

[c]Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisboa, Lisboa, Caparica, Portugal

Patrícia Poeta[a,b,*]

[a]MicroART - Microbiology and Antibiotic Resistance Team, Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal

[b]Associated Laboratory for Green Chemistry (LAQV-REQUIMTE), University NOVA of Lisboa, Lisboa, Caparica, Portugal

* Corresponding author. Present address: MicroART - Microbiology and Antibiotic Resistance Team, University of Trás-os-Montes and Alto Douro, 5000-911, Vila Real, Portugal.
*E-mail address:* ppoeta@utad.pt (P. Poeta).

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