Supplementary Material

Detection of *cfr*-positive MRSA CC398 in a pig farmer in Spain

Table S1. Primer pairs used for the molecular typing and detection of antimicrobial resistance genes, mutations and virulence determinants in the *S. aureus* isolate.

Target gene	**Primer sequence (5’-3’)**	**Amplicon size (bp)**	**Reference**
Antimicrobial resistance			
mecA	F: GGGATCATAGCGTCATTATTC R: AACGATTGTGACACGATAGCC	527	1
mecB	F: TTAACATATACACCCGCTTG R: TAAAGTTCATTAGGCACCTCC	527	2
mecC	F: GCTCCTAATGCTAATGCA R: TAAGCAATAATGACTACC	304	3
blaZ	F: CAGTTCACATGCCAAAGAG R: TACACTCTTGGCGGTTTC	772	4
lnu(A/A’)	F: GGTGGCTGGGGGGTAGATGTATTAACTGG R: GCTTCTTTTGAAATACATGGTATTTTTCGATC	322	5
lnu(B)	F: CCTACCTATTGTTTGTGGAA R: ATAACGTTACTCTCCTATTC	944	6
lsa(B)	F: TGCCGAAGCCATGTTACCGTCC R: CGGTTAGACCAACCAGCCGAACG	396	7
vga(A)	F: AGTGGTGGTGAAGTAACACG R: GGTTCAATACTCAATCGACTGAG	1264	8
tet(K)	F: TTAGGTGAAGGGTTAGGTCC R: GCAAACTCATTCCAGAAGCA	697	9
tet(L)	F: CATTTGGTCTTATTGGATCG R: ATTACACTTCCGATTTCGG	456	
tet(M)	F: GTTAAATAGTGTTCTTGGAG	576	

	R: CTAAGATATGGCTCTAACAA		
fexA	F: GTACTTGTAGGTGCAATTACGGCTGA	1272	10
	R: CGCATCTGAGTAGGACATAGCGTC		
fexB	F: TTCCCACTATTGGTGAAAGGAT	816	11
	R: GCAATTCCCTTTTATGGACGTT		
cat$_{\text{pC194}}$	F: CGACTTTTAGTATAACCACAGA	570	4
	R: GCCAGTCATTAGGCCTAT		
cat$_{\text{pC221}}$	F: ATTTATGCAATTATGGAAGTTG	434	
	R: TGAAGCATGGTAACCATCAC		
cat$_{\text{pC223}}$	F: GAATCAAATGCTAGTTTTAACTC	283	
	R: ACATGGTAACCATCACATAC		
ant(4')-Ia	F: GCAAGGACCGACAACATTTC	165	12
	R: TGGCACAGATGGTCATAACC		
aac(6')-Ie-*aph*(2")-Ia	F: CCAAGAGCAATAAGGGCATA	220	
	R: CACTATCATAACCACTACCG		
cfr	F: TGAAGTATAAAGCAGGTTGGGAGTCA	746	13
	R: ACCATATAATTGACCACAAGCAGC		
cfr(B)	F: TGAGCATATACGAGTAACCTCAAGA	293	14
	R: CGCAAGCAGCGTCTATATCA		
optrA	F: AGGTGGTCAGCGAACTAA	1395	15
	R: ATCA ACTGTTCCCATTCA		
poxtA	F: TCAATGCAGAGCAGGAAGCA	791	16
	R: GGTGGATTTACCGACACCGT		
Mutations			
23S rRNA	F: GCGGTCGCCTCCTAAAAG	420	17

	R: ATCCCGGTCCTCTCGTACT		
L3 (*rplC*)	F: ACCCTGATTTAGTTCCGTCTA	799	18
	R: GTTGACGCTTTAATGGGCTTA		
L4 (*rplD*)	F: TCGCTTACCTCCTTAATG	1080	
	R: GGTGGAAACACTGTAACTG		
L22 (*rplV*)	F: CAACACGAAGTCCGATTGGA	468	19
	R: GCAGACGACAAGAAAACAAG		
gyrA	F: AATGAACAAGGTATGACACC	222	20
	R: TACGCGCTTCAGTATAACGC		
grlA	F: ACTTGAAGATGTTTTAGGTGAT	559	
	R: TTAGGAAATCTTGATGGCAA		
Molecular typing			
spa	F: AGACGATCCTTCGGTGAGC	Variable	21
	R: GCTTTTGCAATGTCATTTACTG		
arcC	F: TTGATTCACCAGCGCGTATTGTC	456	22
	R: AGGTATCTGCTTCAATCAGCG		
aroE	F: ATCGGAAATCCTATTTCACATTC	456	
	R: GGTGTTGTATTAATAACGATATC		
glpF	F: CTAGGAACTGCAATCTTAATCC	465	
	R: TGGTAAAATCGCATGTCCAATTC		
gmk	F: ATCGTTTTATCGGGACCATC	429	
	R: TCATTAACTACAACGTAATCGTA		
pta	F: GTTAAAATCGTATTACCTGAAGG	474	
	R: GACCCTTTTGTTGAAAAGCTTAA		
tpi	F: TCGTTCATTCTGAACGTCGTGAA	402	

	R: TTTGCACCTTCTAACAATTGTAC		
yquiL	F: CAGCATACAGGACACCTATTGGC R: CGTTGAGGAATCGATACTGGAAC	516	
*agr*I	F: GTCACAAGTACTATAAGCTGCGAT R: ATGCACATGGTGCACATGC	440	23
*agr*II	F: GTATTACTAATTGAAAAGTGCCATAGC R: ATGCACATGGTGCACATGC	572	
*agr*III	F: CTGTTGAAAAAGTCAACTAAAAGCTC R: ATGCACATGGTGCACATGC	406	
*agr*IV	F: CGATAATGCCGTAATACCCG R: ATGCACATGGTGCACATGC	656	
SCC*mec* V	F: GAACATTGTTACTTAAATGAGCG R: TGAAAGTTGTACCCTTGACACC	325	24
Virulence			
lukF/S-PV	F: ATCATTAGGTAAAATGTCTGGACATGATCCA R: GCATCAAGTGTATTGGATAGCAAAAGC	443	5
tst	F: TTCACTATTTGTAAAAGTGTCAGACCCACT R: TACTAATGAATTTTTTTATCGTAAGCCCTT	180	25
eta	F: ACTGTAGGAGCTAGTGCATTTGT R: TGGATACTTTTGTCTATCTTTTTCATCAAC	190	
etb	F: CAGATAAAGAGCTTTATACACACATTAC R: AGTGAACTTATCTTTCTATTGAAAAACACTC	612	
Immune Evasion Cluster (IEC)			
scn	F: AGCACAAGCTTGCCAACATCG R: TTAATATTTACTTTTTAGTGC	257	26

chp	F: TTTACTTTTGAACCGTTTCCTAC	366
	R: CGTCCTGAATTCTTAGTATGCATATTCATTAG	
sak	F: AAGGCGATGACGCGAGTTAT	223
	R: GCGCTTGGATCTAATTCAAC	
sea	F: AGATCATTCGTGGTATAACG	344
	R: TTAACCGAAGGTTCTGTAGA	
sep	F: AATCATAACCAACCGAATCA	196
	R: TCATAATGGAAGTGCTATAA	

Table S2. Primer pairs used to determine the genetic environment of the *cfr* gene.

Primer	**Sequence (5'-3')**	**Reference**
cfrinv-F	TGACTCCCAACCTGCTTTATACTTCA	27
cfrinv-R	GCTGTTGTGGTCAATTATATGGT	
fexAinv-R	GACGCTATGTCCTACTCAGATGCG	
pSCFS7-F	GTACTGTAACTGGACGAATATCTAC	
tnpB-2-F	GGAACAGTGATAAGCCGGATGAT	
tnpB-2-R	GAAGGTGTATCCTTCCAGTCATATC	
cfr-ent-1-F	CTGTCAATTTCCTCAAACAG	
cfr-ent-1-R	ATTAATACGTCCTCTGAACG	
istAS-F	GAGATTGTGGAAAGGCTCAAGG	This study
istAS-R	TGTTCAAGCACTCCTTCATCGAC	
istBS-F	GAATTACGTCTTCCTGGCATCC	
istBS-R	GTAAGATGGGCCGATCATATTCA	
pSCFS3-F	GGTGGTGGAAAGATGAAGGTTCAG	
pSCFS3-R	GTGAAGACCGCCAGTGAATAGGAC	

References

1. Poulsen AB, Skov R, Pallesen LV. Detection of methicillin resistance in coagulase-negative staphylococci and in staphylococci directly from simulated blood cultures using the EVIGENE MRSA Detection Kit. J Antimicrob Chemother 2009;**51**:419-21. https://doi.org/10.1093/jac/dkg084.
2. Becker K, van Alen S, Idelevich EA, Schleimer N, Seggewiß J, Mellmann A, et al. Plasmid-encoded transferable *mecB*-mediated methicillin resistance in *Staphylococcus aureus*. Emerg Infect Dis 2018;**24**:242-8. https://doi.org/10.3201/eid2402.171074.
3. Cuny C, Layer F, Strommenger B, Witte W. Rare occurrence of methicillin-resistant *Staphylococcus aureus* CC130 with a novel *mecA* homologue in humans in Germany. PLoS ONE 2011;**6**:e24360. https://doi.org/10.1371/journal.pone.0024360.
4. Schnellmann C, Gerber V, Rossano A, Jaquier V, Panchaud Y, Doherr MG, et al. Presence of new *mecA* and *mph*(C) variants conferring antibiotic resistance in *Staphylococcus* spp. isolated from the skin of horses before and after clinic admission. J Clin Microbiol 2011;**44**:4444-54. https://doi.org/10.1128/JCM.00868-06.
5. Lina G, Piémont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, et al. Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin Infect Dis 1999;**29**:1128-32. https://doi.org/10.1086/313461.

6. Bozdogan B, Berrezouga L, Kuo MS, Yurek DA, Farley KA, Stockman BJ, Leclercq R. A new resistance gene, *linB*, conferring resistance to lincosamides by nucleotidylation in *Enterococcus faecium* HM1025. Antimicrob Agents Chemother 1999;**43**:925-9.
7. Gómez-Sanz E, Torres C, Lozano C, Fernández-Pérez R, Aspiroz C, Ruiz-Larrea F, et al. Detection, molecular characterization, and clonal diversity of methicillin-resistant *Staphylococcus aureus* CC398 and CC97 in Spanish slaughter pigs of different age groups. Foodborne Pathog Dis 2010;**7**:1269-77. https://doi.org/10.1089/fpd.2010.0610.
8. Lozano C, Aspiroz C, Rezusta A, Gómez-Sanz E, Simon C, Gómez P, et al. Identification of novel *vga*(A)-carrying plasmids and a Tn*5406*-like transposon in methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis* of human and animal origin. Int J Antimicrob Agents 2012;**40**:306-12. https://doi.org/10.1016/j.ijantimicag.2012.06.009.
9. Aarestrup FM, Agerso Y, Gerner-Smidt P, Madsen M, Jensen LB. Comparison of antimicrobial resistance phenotypes and resistance genes in *Enterococcus faecalis* and *Enterococcus faecium* from humans in the community, broilers, and pigs in Denmark. Diagn Microbiol Infect Dis 2000;**37**:127-37. https://doi.org/10.1016/s0732-8893(00)00130-9.
10. Kehrenberg C, Schwarz S. Florfenicol-chloramphenicol exporter gene *fexA* is part of the novel transposon Tn*558*. Antimicrob Agents Chemother 2005;**49**:813-5. https://doi.org/10.1128/AAC.49.2.813-815.2005.
11. Liu H, Wang Y, Wu C, Schwarz S, Shen Z, Jeon B, et al. A novel phenicol exporter gene, *fexB*, found in enterococci of animal origin. J Antimicrob Chemother 2012;**67**:322-5. https://doi.org/10.1093/jac/dkr481.

12. van de Klundert JAM, Vliegenthart JS. PCR detection of genes coding for aminoglycoside-modifying enzymes. In: Persing DH, Smith TF, Tenover FC, Wite TJ, editors. *Diagnostic Molecular Microbiology. Principles and applications*, Washington: ASM; 1993.

13. Kehrenberg C, Schwarz S. Distribution of florfenicol resistance genes *fexA* and *cfr* among chloramphenicol-resistant *Staphylococcus* isolates. Antimicrob Agents Chemother 2006;**50**:1156-63. https://doi.org/10.1128/AAC.50.4.1156-1163.2006.

14. Lee SM, Huh HJ, Song DJ, Shim HJ, Park KS, Kang CI, et al. Resistance mechanisms of linezolid-nonsusceptible enterococci in Korea: low rate of 23S rRNA mutations in *Enterococcus faecium*. J Med Microbiol. 2017;**66**:1730-5. https://doi.org/10.1099/jmm.0.000637.

15. Wang Y, Lv Y, Cai J, Schwarz S, Cui L, Hu Z, et al. A novel gene, *optrA*, that confers transferable resistance to oxazolidinones and phenicols and its presence in *Enterococcus faecalis* and *Enterococcus faecium* of human and animal origin. J Antimicrob Chemother 2015;**70**:2182-90. https://doi.org/10.1093/jac/dkv116.

16. Ruiz-Ripa L, Feßler AT, Hanke D, Sanz S, Olarte C, Mama OM, et al. Coagulase-negative staphylococci carrying *cfr* and PVL genes, and MRSA/MSSA-CC398 in the swine farm environment. Submitted.

17. Dibo I, Pillai SK, Gold HS, Baer MR, Wetzler M, Slack JL, et al. Linezolid-resistant *Enterococcus faecalis* isolated from a cord blood transplant recipient. J Clin Microbiol 2000;**42**:1843-5. https://doi.org/10.1128/jcm.42.4.1843-1845.2004.

18. Miller K, Dunsmore CJ, Fishwick CW, Chopra I. Linezolid and tiamulin cross-resistance in *Staphylococcus aureus* mediated by point mutations in the peptidyl transferase center. Antimicrob Agents Chemother 2008;**52**:1737-42. https://doi.org/10.1128/AAC.01015-07.

19. Mendes RE, Deshpande LM, Farrell DJ, Spanu T, Fadda G, Jones RN. Assessment of linezolid resistance mechanisms among *Staphylococcus epidermidis* causing bacteraemia in Rome, Italy. J Antimicrob Chemother 2010;**65**:2329-35. https://doi.org/10.1093/jac/dkq331.

20. Schmitz FJ, Hofmann B, Hansen B, Scheuring S, Lückefahr M, Klootwijk M, et al. Relationship between ciprofloxacin, ofloxacin, levofloxacin, sparfloxacin and moxifloxacin (BAY 12-8039) MICs and mutations in *grlA*, *grlB*, *gyrA* and *gyrB* in 116 unrelated clinical isolates of *Staphylococcus aureus*. J Antimicrob Chemother 1998;**41**:481-4. https://doi.org/10.1093/jac/41.4.481.

21. Shopsin B, Gomez M, Montgomery SO, Smith DH, Waddington M, Dodge DE, et al. Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. J Clin Microbiol 1999;**37**:3556-63.

22. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J Clin Microbiol 2000;**38**:1008-15.

23. Shopsin B, Mathema B, Alcabes P, Said-Salim B, Lina G, Matsuka A, et al. Prevalence of *agr* specificity groups among *Staphylococcus aureus* strains colonizing children and their guardians. J Clin Microbiol 2003;**41**:456-9. https://doi.org/10.1128/jcm.41.1.456-459.2003.

24. Zhang K, McClure JA, Elsayed S, Louie T, Conly JM. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome *mec* types I to V in methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 2005;**43**:5026-33. https://doi.org/10.1128/JCM.43.10.5026-5033.2005.

25. Jarraud S, Mougel C, Thioulouse J, Lina G, Meugnier H, Forey F, et al. Relationships between *Staphylococcus aureus* genetic background, virulence factors, *agr* groups (alleles), and human disease. Infect Immun 2002;**70**:631-41. https://doi.org/10.1128/iai.70.2.631-641.2002.

26. van Wamel WJ, Rooijakkers SH, Ruyken M, van Kessel KP, van Strijp JA. The innate immune modulators staphylococcal complement inhibitor and chemotaxis inhibitory protein of *Staphylococcus aureus* are located on ß-hemolysin-converting bacteriophages. J Bacteriol. 2006;**188**:1310-5. https://doi.org/10.1128/JB.188.4.1310-1315.2006.

27. Lozano C, Ruiz-García M, Gómez-Sanz E, López-García P, Royo-García G, Zarazaga M, et al. Characterization of a *cfr*-positive methicillin-resistant *Staphylococcus epidermidis* strain of the lineage ST22 implicated in a life-threatening human infection. Diagn Microbiol Infect Dis 2012;**73**:380-2. https://doi.org/10.1016/j.diagmicrobio.2012.04.013.