Antimicrobial resistance phenotypes and genotypes of methicillin-resistant *Staphylococcus aureus* CC398 isolates from Spanish hospitals

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Highlights

- A multidrug resistant phenotype was present in 79% of MRSA CC398 strains.
- Almost 20% of strains were resistant to six or more antimicrobial families.
- The *tetM* gene was present in all tetracycline-resistant MRSA CC398 strains.
- Resistance to CIP (67%), ERY/CLI (48%) or GEN/TOB (21%) were common.
- Detection of ERY^{S}-CLI^{R} strains was frequent, with *linA*, *linB*, *lsaB* and *vgaA* genes.

Abstract

Livestock-associated (LA) methicillin-resistant *Staphyloccoccus aureus* (MRSA) of lineage CC398 is an emerging clone causing human infections, although they are most commonly found in pigs. The aim of this study was to characterize the antimicrobial resistance phenotypes/genotypes of a collection of 137 MRSA CC398 isolates obtained in a previous study from 17 Spanish hospitals, using tetracycline resistance as marker for selection. A multidrug resistant (MDR) phenotype was present in 79% of analysed isolates, with 17% of them resistant to six or more different antimicrobial families. All tetracycline resistant isolates (n=137) carried the *tetM* gene and 75% also carried the *tetK* gene. Almost 50% of MRSA CC398 isolates showed macrolide and/or lincosamide resistance: a) 39% of isolates were ERY^R-CLI^R (all with constitutive phenotype), with 87% of them carrying the *ermC* gene, followed by *msrA* (25%), *ermB* (21%), *vgaA* (17%), *ermA* (6%), *lsaB* (4%), *linA* (2%), *linB* (2%), and *erm*T (2%, this isolate with the new *spa*-type t18071); b) 9% of MRSA CC398 isolates showed the dissociated ERY^S-CLI^R phenotype carrying the *linA*, *linB*, *lsaB* and *vgaA* genes. Other antimicrobial resistance phenotypes were present in these MRSA CC398 isolates, such as resistance to ciprofloxacin (67%), aminoglycosides (21%), mupirocin (6%), chloramphenicol (4%) or fusidic acid (2%). The more common resistance genes detected for some of these antimicrobials were: *aac(6')-Ie-aph(2'')-Ia* (16%) and *ant(4')-Ia* (12%) for aminoglycosides, and *fexA* (3%) for chloramphenicol. The high rate of MDR phenotypes with a wide range of antimicrobial resistance genes shown in this study reduce the potential therapeutic options in case of infections.

Keywords: LA-MRSA, CC398, antimicrobial resistance, MDR phenotype, multicentre study.

1. Introduction

Methicillin-resistant *Stapyloccoccus aureus* (MRSA) is considered one of the major therapeutic problems due to its difficulty when treating infections [1]. MRSA clones are widely spread in hospitals and in the community. Livestock-associated MRSA (LA-MRSA) clones are emerging as cause of human infections. The LA-MRSA CC398 clone is commonly found in pigs; however, this genetic lineage has been transmitted from pigs to humans with subsequent human colonization and infection [2].

Tetracycline is one of the most used antimicrobials in food production animals in Europe according to the European Medicines Agency, and the use of this agent might have contributed to the acquisition of tetracycline resistance (TET^R) in some LA-MRSA genetic lineages [3]. In fact, one of the most outstanding phenotypic features of LA-MRSA CC398 lineage is the TET^R phenotype, which is commonly used as a marker for its detection [4].

Mobile genetic elements (MGEs) involve 15-20% of *S. aureus* genome, including bacteriophages, SCC*mec* elements, plasmids or transposons [5]. MGEs can carry resistance and virulence genes as well as host-specific factors that could be important for this pathogen adaptation to new hosts [6]. Pig-associated CC398 isolates usually present β-lactam antibiotic resistance (SCC*mec* elements) and tetracycline resistance (*tetM* gene), with absence of the human immune evasion cluster (IEC) genes (located in ϕSa3int prophage). On the other hand, human-associated *S. aureus* CC398 strains usually are methicillin and tetracycline susceptible and carry the human IEC genes [7]. Resistance of MRSA CC398 strains to other antimicrobial classes, such as macrolides, lincosamides or trimethoprim/sulfamethoxazole, is frequent [8]. The most frequent mechanism of resistance to macrolides is by the production of Erm methylases (encoded by *erm* genes) that confers resistance to both macrolides (erythromycin: ERY) and lincosamides (clindamycin: CLI) (phenotype ERY^R-CLI^R); nevertheless, specific

mechanisms of resistance for lincosamides, seems to be emerging among MRSA CC398 clone (and very unusual in other MRSA clones) (phenotype ERY^S-CLI^R) [4].

Contact with pigs is a well-known risk factor for human MRSA CC398 colonization or infection, with pig farmers and veterinarians as the main hosts due to the direct animal contact [9]. MRSA CC398 isolates found in humans with animal contact share the same characteristics as those found in animals (*tetM* positive and IEC negative). However, in recent years the number of colonization cases with MRSA CC398 have been increasing in people without direct contact with animals, supporting the theory of a subsequent possible human to human transmission or additional routes of dissemination [4]. In 2007, the first MRSA CC398 hospital outbreak was detected [10], suggesting new ways of transmission.

A multicentre study has been recently performed by our group to determine the prevalence of MRSA CC398 genetic lineage in 20 Spanish hospitals of 13 different geographic regions, obtaining MRSA CC398 isolates in 17 of them. A statistically significant correlation was evidenced between the MRSA-CC398 prevalence at hospitals and the pig-farming density of the surrounding regions [11]. The aim of the present study was to characterize the antimicrobial resistance phenotypes/genotypes of the recovered collection of MRSA CC398 isolates.

2. Methods

2.1. Selection of strains and molecular typing

A collection of 137 MRSA CC398 isolates, obtained from 17 Spanish hospitals in a previous work [11], was analyzed in the present study. To obtain this collection, all TET^R MRSA isolates recovered during January-June 2016 in the hospitals were tested, and those corresponding to lineage CC398 were included in the present study. These MRSA CC398

137 isolates were obtained from clinical (74.5%) and epidemiological surveillance (25.5%)
138 samples. The clinical samples corresponded to skin and soft tissue infections (SSTI, n=52),
139 respiratory tract infections (RTI, n=24), surgical site infections (n=15), urinary tract infections
140 (n=6) and blood (n= 5). The epidemiological surveillance samples corresponded in most of
141 the cases (>85%) to nasal samples.

142 The IEC system was analysed in the previous study as well as the *spa*-type, determining that
143 72% of isolates corresponded to spa-t011 [11]. The sequence type (ST) of new *spa*-types was
144 determined by multilocus sequence typing (MLST) in the present study, as previously
145 described (https://pubmlst.org).

146 *2.2.Antimicrobial resistance phenotypes and genotypes*

147 The resistance phenotype to 13 antimicrobial agents, other than β-lactams and tetracycline, as
148 erythromycin, clindamycin, ciprofloxacin, trimethoprim/sulfamethoxazole, vancomycin,
149 teicoplanin, linezolid, daptomycin, fusidic acid, mupirocin, gentamicin, tobramycin, and
150 chloramphenicol, was performed using automatic methods and/or disk diffusion test.
151 Breakpoints were considered according to the Clinical and Laboratory Standards Institute
152 and/or the European Committee on Antimicrobial Susceptibility Testing, depending on
153 hospitals. PCR technique was used for the detection of 25 antimicrobial resistance genes
154 (antimicrobial families to which they confer resistance): *tetM, tetK,* and *tetL* (tetracycline)*;*
155 *blaZ* (penicillin)*; ermA, ermB, ermC, ermT, msrA, linA, linB, lsaB,* and *vgaA* (macrolides
156 and/or lincosamides)*, ant(4')-Ia,* and *aac(6')-Ie-aph(2'')-Ia* (aminoglycosides)*, fexA, fexB,*
157 cat_{pC194}*,* cat_{pC221}*,* and cat_{pC223} (phenicols)*, cfr* (lincosamides, streptogramines, phenicols, and
158 oxazolidinones)*, optrA* (phenicols and oxazolidinones), *poxtA* (tetracyclines, phenicols, and
159 oxazolidinones), *mupA* (mupirocin), *fusB* (fusidic acid) [4,12]. The presence of *mecA* gene
160 was previously determined [11]. Positive and negative controls from the University of La

Rioja were used in each PCR. A multidrug resistant (MDR) phenotype was considered when the isolate was resistant to, at least, one antimicrobial agent of three or more different antimicrobial families. Statistical analyses were performed using the RStudio program (version 1.1.453) ($p<0.01$ was considered statistically significant).

3. Results

3.1.Multidrug resistant (MDR) phenotypes

Resistance to several groups of antimicrobials was detected in the collection of MRSA CC398 isolates (Figure 1, Table S1). A MDR phenotype was present in 78.8% (108/137) of isolates, with 31.5% (34/108) of them resistant to three different families, 20.4% (22/108) resistant to four families, 31.5% (34/108) resistant to five families, and 16.7% (18/108) resistant to six or more different families of drugs (Table S1). None of the isolates presented resistance to vancomycin, teicoplanin, linezolid or daptomycin.

Regarding the origin of isolates, 85% of clinical isolates presented a MDR phenotype whereas 60% of epidemiologic surveillance isolates were MDR ($p<0.01$). Considering epidemiological surveillance isolates, 14% of them showed resistance to mupirocin or fusidic acid.

3.2.Tetracycline resistance genotypes

As tetracycline resistance was used as phenotypic marker for the selection of strains, all isolates carried at least one gene responsible for this resistance. All MRSA CC398 were positive for the *tetM* gene (Figure 2). In addition, 75% of isolayes also carried the *tetK* gene and 6% the *tetL* gene. The most frequent combination of *tet* genes within the MRSA CC398 collection was *tetM* with *tetK* (69%), followed by *tetM* by itself (25%). Seven isolates showed the combination *tetM*, *tetK* and *tetL*, and only one isolate was detected with *tetM* and *tetL* together.

184 *3.3. Macrolide and lincosamide resistance phenotypes and genotypes*

185 Regarding macrolides and lincosamides, 47.5% (65/137) of MRSA CC398 isolates showed a
186 resistant phenotype (Figure 1): a) fifty-three isolates (38.7%) presented an ERY^{R}-CLI^{R}
187 resistance phenotype, and in all cases, clindamycin resistance was constitutive; b) twelve
188 additional isolates (8.8%) showed an ERY^{S}-CLI^{R} resistance phenotype. The genes responsible
189 of macrolide and lincosamide resistances are shown in Figure 2. For ERY^{R}-CLI^{R} isolates, the
190 detected resistance genes were: *ermC* (46/53), *msrA* (13/53), *ermB* (11/53), *vgaA* (9/53), *ermA*
191 (3/53), *lsaB* (2/53), *linA* (1/53), *linB* (1/53), and *ermT* (1/53). On the other hand, for ERY^{S}-
192 CLI^{R} isolates the genes detected were: *linB* (7/12), *linA* (4/12), *vgaA* (3/12), and *lsaB* (2/12).

193 *3.4.Aminoglycoside resistance phenotypes and genotypes*

194 Twenty-nine MRSA CC398 isolates (21.2%) showed resistance to the aminoglycoside group,
195 including tobramycin in all cases (Figure 1). The combination of gentamicin and tobramycin
196 resistance was present in 76% of resistant isolates, with the remaining strains resistant only to
197 tobramycin (24%). The aminoglycoside resistance genes detected among MRSA CC398
198 isolates were *aac(6')-Ie-aph(2'')-Ia* (76%), and *ant(4')-Ia* (55%) (Figure 2).

199 *3.5. Other antimicrobial resistance phenotypes and genotypes*

200 All MRSA CC398 strains carried the *mecA* gene [11]. In addition, 75% of isolates carried the
201 penicillinase-encoding *blaZ* gene (Figure 2). The studied MRSA CC398 isolates also were
202 resistant to other antimicrobial classes (Figure 1), with 67% of isolates showing ciprofloxacin
203 resistance. Resistance to mupirocin, chloramphenicol and fusidic acid were in the range of 2-
204 6% (Figure 1). The *fexA* gene was responsible for chloramphenicol resistance in 80% of
205 isolates; *mupA* gene was present in 12.5% of mupirocin resistant isolates, and the three fusidic
206 acid-resistant isolates lacked the *fusB* gene.

3.6. Relationship between spa-types and antimicrobial resistant phenotypes and genotypes

Table 1 shows the antimicrobial resistance phenotypes and genotypes according to the *spa*-types previously detected, as well as the summary of resistant isolates for each antimicrobial agent tested and genes detected. The isolate with a new *spa*-type (t18071) was the only one carrying the *ermT* gene, and was ascribed to ST398. Regarding the human IEC genes, all but two isolates lacked this cluster. The IEC type E was detected in a MRSA t011 isolate recovered from a RTI and it was resistant only to tetracycline (*tetM*, *tetK*), in addition to β-lactams (*mec*A, *blaZ*). The IEC type B was present in a MRSA t1939 isolate recovered from a SSTI, with a MDR phenotype (gentamicin-tobramycin-clindamycin-erythromycin-ciprofloxacin-tetracyline) and genotype (*msrA*, *ermB*, *ermC*, *aac(6')-Ie-aph(2'')-Ia*, and *ant(4')-Ia*).

4. Discussion

Colonization or infection caused by MRSA CC398 lineage is common mostly in pig farmers, veterinarians and other livestock associated professionals. The emerging MRSA CC398 infections, not directly related to pig contact but in areas with high pig-farming densities, are becoming more regular [4]. A common feature of MRSA CC398 genetic lineage is its frequent resistance to a wide spectrum of antimicrobials.

In this study, more than 75% of MRSA CC398 isolates showed a MDR phenotype, supporting previous studies with clinical MRSA CC398 isolates [4]. Moreover, at least 15% of these isolates was found resistant to six or more different antimicrobial families. This fact makes the election of an effective antimicrobial treatment a difficult challenge.

Regarding tetracycline, the *tetM* gene was detected in all MRSA CC398 isolates analysed, alone or in combination with other *tet* genes. The *tetM* gene encodes a ribosome protection

protein, in charge of reducing the ribosome affinity to tetracycline (in presence of GTP), to avoid the inhibition of protein synthesis by tetracycline [13]. In addition to *tetM*, 75% of our isolates carried the *tetK* gene, which is known to integrate in the SCC*mec* element and is, therefore, genetically linked to the *mecA* gene. Because co-presence of *tetM* and *tetK* increases fitness under tetracycline exposure, this compound can lead to the spread of MRSA by co-selection of the *tetK* and the *mecA* genes [3]. As the presence of the *tetM* gene and the absence of the IEC system are considered defining features of livestock-associated MRSA CC398 [7], all MRSA CC398 isolates studied point to an animal origin. The exception is a couple of MRSA CC398 isolates detected carrying both *tetM* and IEC genes. These two isolates could be part of a possible process of re-adaptation of the MRSA CC398 genetic lineage to the human host [11], posing a threat to public health due to the evolutionary advantage that facilitates colonization and infection in humans.

Macrolide-lincosamide resistance due to more than one *erm* gene is common among MRSA CC398 isolates. In this study, 48% of isolates were macrolide and/or lincosamide resistant, consistent with data found in previous studies [14]. The most disseminated *erm* gene within the *Staphylococcus* genus is *ermC*, mostly found within plasmids [15], and it was detected in 87% of the ERY^R-CLI^R analysed isolates (or 34% of total MRSA CC398). The only isolate carrying the *ermT* gene (with the new *spa*-type t18071), also presented many other resistance genes (*tetM*, *tetK*, *tetL*, *ermC*, *aac(6')-Ie-aph(2'')-Ia,* and *ant(4')-Ia*). The *ermT* gene has been described usually in MSSA CC398 isolates of human origin, but they are uncommon in LA-MRSA strains [16,17], or in hospital- or community-associated MRSA isolates. This gene can be found both in plasmids or in the chromosomal DNA [18]. The *msrA* gene is not common in the MRSA CC398 genetic lineage [8], with 10% of *msrA*-positive isolates in our MRSA CC398 collection.

Resistance to lincosamides associated to macrolides susceptibility (ERY^S-CLI^R phenotype) is very unusual among MRSA of human origin, although is more frequent among MRSA isolates of animal origin [19], and in our study almost 10% of the total analysed isolates had this particular phenotype (with *linA*, *linB*, *lsaB* or *vgaA* genes). The absence of MRSA CC398 isolates showing the ERY^R-CLI^R phenotype with inducible clindamycin resistance is of note, since this phenotype was frequently found in other human-linked MRSA and MSSA genetic lineages.

Aminoglycoside resistance is also frequent in staphylococcal species [19]. Specifically, 21% of analysed isolates showed resistance to this antimicrobial family, and in all cases, isolates were tobramycin resistant (alone or in combination with gentamicin).

Other antimicrobial resistance phenotypes can be present in MRSA CC398 isolates, with 67% of isolates resistant to ciprofloxacin. Slightly lower rates of resistance (51-57%) have been found in previous studies in Spain [4,20], caused by amino acid changes in the quinolone targets, GrlA/ GyrA, with S80F/S84L changes as the most frequent ones [20].

Tetracycline, macrolides, lincosamides or fluoroquinolones are important antimicrobial treatment choices for SSTIs or RTIs. In our study, 74% of clinical MRSA CC398 isolates were obtained from SSTIs and RTIs [11], 67% were fluoroquinolone-resistant, and almost 50% macrolide and/or lincosamide-resistant. On the other hand, the rates of resistance to mupirocin and fusidic acid, antimicrobials frequently used for MRSA decolonization, were 6% and 2%, respectively.

In conclusion, the capability of MRSA CC398 of acquiring a great amount of antimicrobial resistance genes makes this livestock-associated genetic lineage a case of public health concern, what poses a great challenge in terms of selection of accurate clinical therapies, in

case of infections. Some phenotypic markers could alert microbiologists about the presence of this clone: TET^R (always detected among MRSA CC398, but infrequent in other clones), and ERY^S-CLI^R (relatively frequent among MRSA CC398, and really uncommon in other clones). Surveillance of this specific MRSA lineage, especially in areas with high pig farming density, would be required.

Declarations

Funding: This work was supported by the Agencia Estatal de Investigación (AEI) of Spain (project SAF2016-76571-R) and the Fondo Europeo de Desarrollo Regional (FEDER) of EU, and also by the Sociedad de Enfermedades Infecciosas del Norte (SEINORTE). Sara Ceballos and Laura Ruiz-Ripa have predoctoral fellowships of the University of La Rioja (Spain) during the experimental part of the study.

Part of this work was presented as a poster (P1727) in the 28th ECCMID, Madrid, Spain, and as an oral communication (number 1046) in the 22nd SEIMC, Bilbao, Spain.

Competing interests: None declared.

Ethical approval: Not required.

Author contribution: All authors have approved the final article and have made substantial contributions to all of the following: CT and CA conceived and designed the study; the study group supplied the MRSA strain collection; SC and LRR performed laboratory works; SC, CA, MZ and CT interpreted the results; SC, CA and CT drafted the article or revised it critically for important intellectual content. All co-authors and the study group revised and approved the version to be submitted to the journal.

References

[1] Bassetti M, Poulakou G, Ruppe E, Bouza E, Van Hal SJ, Brink A. Antimicrobial resistance in the next 30 years, humankind, bugs and drugs: a visionary approach. Intensive Care Med 2017;43:1464–75. doi:10.1007/s00134-017-4878-x.

[2] Verkade E, Kluytmans-van Den Bergh M, Van Benthem B, Van Cleef B, Van Rijen M, Bosch T, et al. Transmission of methicillin-resistant *Staphylococcus aureus* CC398 from livestock veterinarians to their household members. PLoS One 2014;9. doi:10.1371/journal.pone.0100823.

[3] Larsen J, Clasen J, Hansen JE, Paulander W, Petersen A, Larsen AR, et al. Copresence of *tet*(K) and *tet*(M) in livestock-associated methicillin-resistant S*taphylococcus aureus* clonal complex 398 is associated with increased fitness during exposure to sublethal concentrations of tetracycline. Antimicrob Agents Chemother 2016;60:4401–3. doi:10.1128/AAC.00426-16.Address.

[4] Benito D, Lozano C, Rezusta A, Ferrer I, Vasquez MA, Ceballos S, et al. Characterization of tetracycline and methicillin resistant *Staphylococcus aureus* strains in a Spanish hospital: Is livestock-contact a risk factor in infections caused by MRSA CC398? Int J Med Microbiol 2014;304:1226–32. doi:10.1016/j.ijmm.2014.09.004.

[5] Lindsay JA. *Staphylococcus aureus* genomics and the impact of horizontal gene transfer. Int J Med Microbiol 2014;304:103–9. doi:10.1016/j.ijmm.2013.11.010.

[6] McCarthy AJ, Loeffler A, Witney AA, Gould KA, Lloyd DH, Lindsay JA. Extensive horizontal gene transfer during *Staphylococcus aureus* co-colonization *in vivo*. Genome Biol Evol 2014;6:2697–708. doi:10.1093/gbe/evu214.

[7] Price LB, Stegger M, Hasman H, Aziz M, Larsen J, Andersen S, et al. *Staphylococcus aureus* CC398: Host adaptation and emergence of methicillin resistance in livestock lance. MBio 2012;3:1–6. doi:10.1128/mBio.00305-11.Editor.

[8] Argudín MA, Tenhagen BA, Fetsch A, Sachsenröder J, Käsbohrer A, Schroeter A, et al. Virulence and resistance determinants of German *Staphylococcus aureus* ST398 isolates from nonhuman sources. Appl Environ Microbiol 2011;77:3052–60. doi:10.1128/AEM.02260-10.

[9] Zarazaga M, Gómez P, Ceballos S, Torres C. Molecular epidemiology of *Staphylococcus aureus* lineages in the animal–human interface. *Staphylococcus aureus,* Elsevier Inc.; 2017, p. 189–214. doi:10.1016/B978-0-12-809671-0.00010-3.

[10] Wulf MW, Markestein A, van der Linden FT, Voss A, Klaassen C, Verduin CM. First outbreak of methicillin-resistant *Staphylococcus aureus* ST398 in a Dutch hospital, June 2007. Eurosurveillance 2008;14:381–4. doi:10.1111/j.1469-0691.2007.01927.x.

[11] Ceballos S, Aspiroz C, Ruiz-Ripa L, Reynaga E, Azcona-Gutiérrez JM, Rezusta A, et al. Epidemiology of MRSA CC398 in hospitals located in Spanish regions with different pig-farming densities: a multicentre study. J Antimicrob Chemother 2019;74:2157–61. doi:10.1093/jac/dkz180.

[12] 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. doi:10.1089/fpd.2010.0610.

[13] Burdett V. *Tet*(M)-promoted release of tetracycline from ribosomes is GTP dependent. J Bacteriol 1996;178:3246–51. doi:10.1128/jb.178.11.3246-3251.1996.

345 [14] Seier-Petersen MA, Nielsen LN, Ingmer H, Aarestrup FM, Agersø Y. Biocide
346 Susceptibility of *Staphylococcus aureus* CC398 and CC30 isolates from pigs and
347 identification of the biocide resistance genes, *qacG* and *qacC*. Microb Drug Resist
348 2015;21:527–36. doi:10.1089/mdr.2014.0215.

349 [15] Feßler AT, Wang Y, Wu C, Schwarz S. Mobile macrolide resistance genes in
350 staphylococci. Plasmid 2018. doi:10.1016/j.plasmid.2018.05.001.

351 [16] Vandendriessche S, Kadlec K, Schwarz S, Denis O. Methicillin-susceptible
352 *Staphylococcus aureus* ST398-t571 harbouring the macrolide-lincosamide-
353 streptogramin B resistance gene *erm*(T) in Belgian hospitals. J Antimicrob Chemother
354 2011;66:2455–9. doi:10.1093/jac/dkr348.

355 [17] Argudín MA, Deplano A, Vandendriessche S, Dodémont M, Nonhoff C, Denis O, et al.
356 CC398 *Staphylococcus aureus* subpopulations in Belgian patients. Eur J Clin
357 Microbiol Infect Dis 2018;37:911–6. doi:10.1007/s10096-018-3205-y.

358 [18] Gómez-Sanz E, Kadlec K, Feßler AT, Zarazaga M, Torres C, Schwarz S. Novel
359 *erm*(T)-carrying multiresistance plasmids from porcine and human isolates of
360 methicillin-resistant *Staphylococcus aureus* ST398 that also harbor cadmium and
361 copper resistance determinants. Antimicrob Agents Chemother 2013;57:3275–82.
362 doi:10.1128/AAC.00171-13.

363 [19] Schwarz S, Feßler AT, Loncaric I, Wu C, Kadlec K, Wang Y, et al. Antimicrobial
364 resistance among staphylococci of animal origin. Microbiol. Spectr., vol. 6, 2018.
365 doi:10.1128/microbiolspec.ARBA-0010-2017.

366 [20] Lozano C, Rezusta A, Gómez P, Gómez-sanz E, Báez N, Martin-saco G, et al. High
367 prevalence of *spa* types associated with the clonal lineage CC398 among tetracycline-

368 resistant methicillin-resistant *Staphylococcus aureus* strains in a Spanish hospital. J
369 Antimicrob Chemother 2012;67:330–4. doi:10.1093/jac/dkr497.

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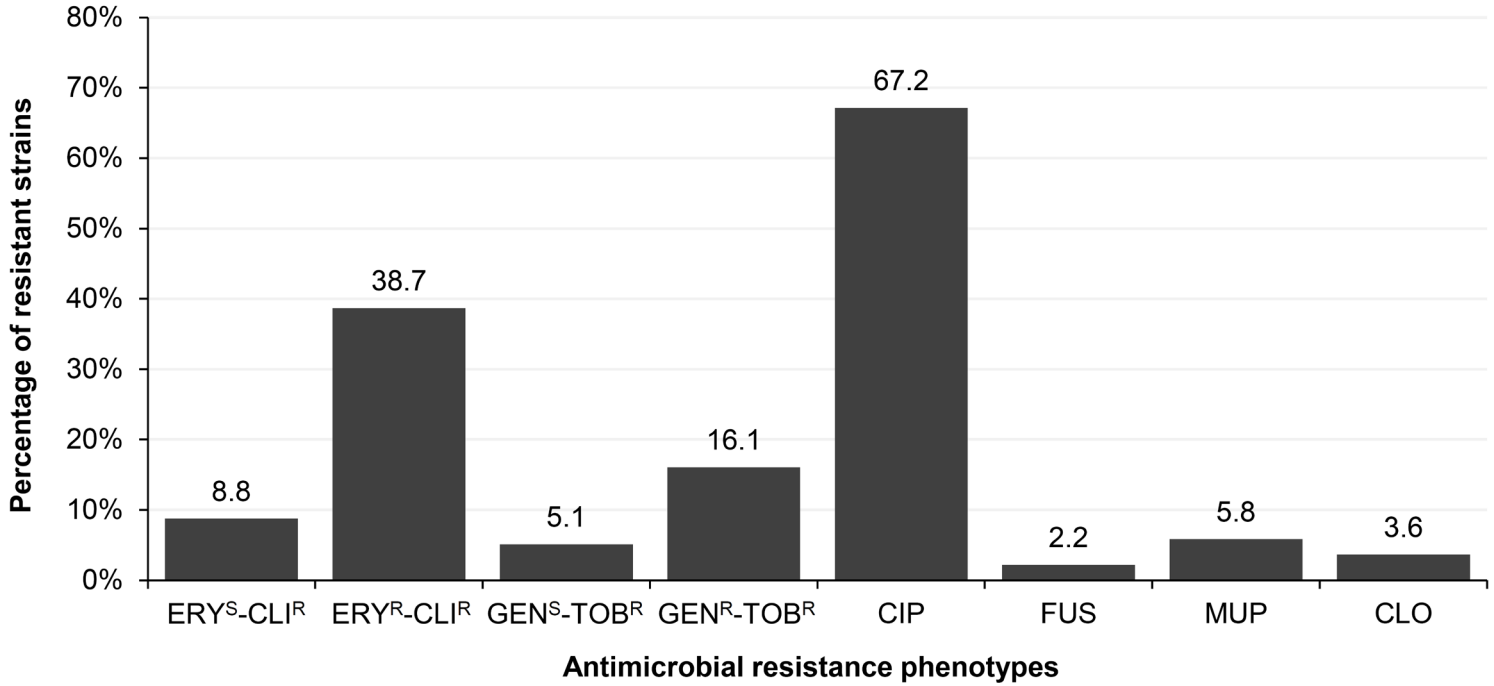
371 Table 1. Antimicrobial resistance phenotypes and genotypes of clinical MRSA CC398 isolates according to their *spa*-types.

spa-type[a]	Resistance phenotype for non-β-lactam agents[a,b]	Antimicrobial resistance genotype[a]
t011[c] (99)	**TET** (99), ERY (36), CLI (47), GEN (13), TOB (19), CIP (62), FUS (2), MUP (6), CLO (3)	***mecA*** (99), ***blaZ*** (73), ***tetM*** (99), ***tetK*** (76), *tetL* (5), *ermA* (1), *ermB* (6), *ermC* (33), *linA* (5), *linB* (6), *lsaB* (4), *vgaA* (9), *aac*(6')-Ie–*aph*(2'')-Ia (13), *ant*(4')-Ia (11), *fexA* (2)
t1451 (10)	TET (10), ERY (5), CLI (5), GEN (4), TOB (4), CIP (7), FUS (1)	*mecA* (10), *blaZ* (8), *tetM* (10), *tetK* (8), *ermB* (1), *ermC* (5), *vgaA* (1), *aac*(6')-Ie–*aph*(2'')-Ia (4), *ant*(4')-Ia (1)
t034 (6)	TET (6), ERY (3), CLI (4), CIP (3), CLO (1)	*mecA* (6), *blaZ* (5), *tetM* (6), *tetK* (6), *ermB* (1), *ermC* (2), *msrA* (1), *linB* (2), *fexA* (1)
t899 (4)	TET (4), ERY (1), CLI (1), TOB (1), CIP (2)	*mecA* (4), *blaZ* (4), *tetM* (4), *tetL*(1), *msrA* (1), *vgaA* (1), *ant*(4')-Ia (1)
t1939[c] (3)	**TET** (3), **ERY** (1), **CLI** (1), **GEN** (3), **TOB** (3), **CIP** (3), MUP (2)	***mecA*** (3), *blaZ* (1), ***tetM*** (3), ***ermB*** (1), ***ermC*** (1), ***msrA*** (1), ***aac*(6')-Ie–*aph*(2'')-Ia** (3), ***ant*(4')-Ia** (1), *mupA* (1)
t108 (2)	TET (2), ERY (1), CLI (1), CIP (2)	*mecA* (2), *blaZ* (1), *tetM* (2), *tetK* (1), *ermA* (1)
t1197 (2)	TET (2), ERY (1), CLI (1), CIP (1)	*mecA* (2), *blaZ* (2), *tetM* (2), *tetK* (1), *tetL* (1), *ermB* (1), *ermC* (1)
t1255 (2)	TET (2), ERY (2), CLI (2), CIP (2)	*mecA* (2), *tetM* (2), *tetK* (2), *ermB* (1), *ermC* (2)
t2346 (2)	TET (2), ERY (1), CLI (1), CIP (1), CLO (1)	*mecA* (2), *blaZ* (2), *tetM* (2), *tetK* (2), *ermC* (1), *fexA* (1)

t1456 (1)	TET (1), ERY (1), CLI (1), CIP (1)	*mecA* (1), *blaZ* (1), *tetM* (1), *tetK* (1), *ermA* (1), *msrA* (1), *vgaA* (1)
t2123 (1)	TET (1), GEN (1), TOB (1)	*mecA* (1), *blaZ* (1), *tetM* (1), *tetK* (1), *aac*(6')-Ie–*aph*(2'')-Ia (1), *ant*(4')-Ia (1)
t2370 (1)	TET (1)	*mecA* (1), *blaZ* (1), *tetM* (1), *tetK* (1)
t2383 (1)	TET (1)	*mecA* (1), *blaZ* (1), *tetM* (1), *tetK* (1)
t2741 (1)	TET (1)	*mecA* (1), *tetM* (1)
t2970 (1)	TET (1), CIP (1)	*mecA* (1), *blaZ* (1), *tetM* (1), *tetK* (1)
t18071 (1)	TET (1), ERY (1), CLI (1), GEN (1), TOB (1), CIP (1)	*mecA* (1), *blaZ* (1), *tetM* (1), *tetK* (1), *tetL* (1), *ermC* (1), *ermT* (1), *aac*(6')-Ie–*aph*(2'')-Ia (1), *ant*(4')-Ia (1)
TOTAL		
16 *spa*-types (137)	TET (137), ERY (53), CLI (65), GEN (22), TOB (29), CIP (92), FUS (3), MUP (8), CLO (5)	*mecA* (137), *blaZ* (102), *tetM* (137), *tetK* (102), *tetL* (8), *ermA* (3), *ermB* (11), *ermC* (46), *ermT* (1), *msrA* (13), *linA* (5), *linB* (8), *lsaB* (4), *vgaA* (12), *aac*(6')-Ie–*aph*(2'')-Ia (22), *ant*(4')-Ia (16), *fexA* (4), *mupA* (1)

[a]Number of isolates in parenthesis.
[b]Antimicrobial agents: TET, tetracycline; ERY, erythromycin; CLI, clindamycin; GEN, gentamicin; TOB, tobramycin; CIP, ciprofloxacin; FUS, fusidic acid; MUP, mupirocin; CLO, chloramphenicol.
[c]IEC positive isolates (one t011 IEC type E and one t1939 IEC type B) with its corresponding antimicrobial resistance phenotype and genotype marked in bold letters.

377 Figure 1. Antimicrobial resistance phenotypes detected in the collection of 137 TET^R-MRSA CC398 isolates. Antimicrobial agents: TET,
378 tetracycline; ERY, erythromycin; CLI, clindamycin; GEN, gentamicin; TOB, tobramycin; CIP, ciprofloxacin; FUS, fusidic acid; MUP,
379 mupirocin; CLO, chloramphenicol. R in superscript: resistant; S in superscript: susceptible.



380

381 Figure 2. Antimicrobial resistance genes detected in the collection of 137 TET^{R}-MRSA CC398 isolates.

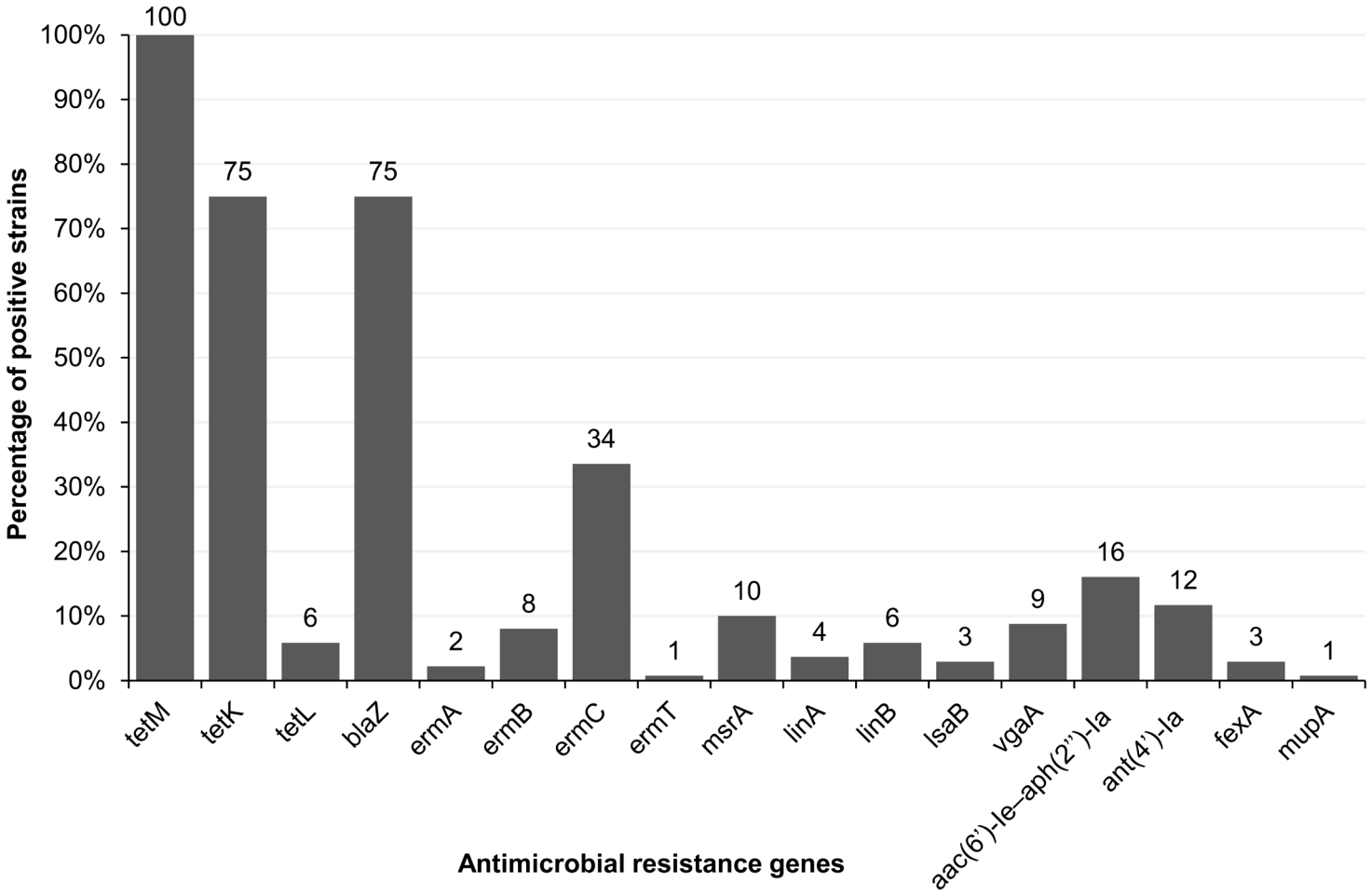


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Table S1. Multidrug resistant (MDR) phenotypes exhibited in the collection of MRSA CC398 isolates with their associated sample origins and *spa*-types.

Different antibiotic families[a]	Phenotype[a,b,c]	Sample origin[c]	*spa*-types[c]
7	GEN-TOB-CLI-ERY-MUP-CIP (2)	Clinical (2)	t011 (2)
6	GEN-TOB-CLI-ERY-CIP (7)	Clinical (5), Epidemiological (2)	t011 (2), t1451 (3), t1939 (1), t18071 (1)
	TOB-CLI-ERY-CIP (5)	Clinical (4), Epidemiological (1)	t011 (3), t899 (1)
	CLI-ERY-CLO-CIP (2)	Clinical (2)	t2346 (1), t034 (1)
	GEN-TOB-CLO-CLI-ERY (1)	Clinical (1)	t011 (1)
	CLI-ERY-CIP-FUS (1)	Epidemiological (1)	t011 (1)
5	CLI-ERY-CIP (24)	Clinical (21), Epidemiological (3)	t011 (18), t034 (1), t108 (1), t1255 (2), t1451 (1), 1456 (1)
	GEN-TOB-CLI-CIP (3)	Clinical (3)	t011 (3)
	GEN-TOB-MUP-CIP (2)	Clinical (2)	t1939 (2)
	GEN-TOB-CIP-FUS (1)	Clinical (1)	t1451 (1)
	GEN-TOB-CLI-ERY (1)	Epidemiological (1)	t011 (1)
	GEN-TOB-CLO-CIP (1)	Clinical (1)	t011 (1)
	CLI-MUP-CIP (1)	Epidemiological (1)	t011 (1)
	TOB-CLI-CIP (1)	Clinical (1)	t011 (1)
4	CLI-ERY (9)	Clinical (7), Epidemiological (2)	t011 (6), t034 (1), t1197 (1), t1451 (1)
	CLI-CIP (6)	Clinical (5), Epidemiological (1)	t011 (5), t034 (1)
	GEN-TOB-CIP (2)	Clinical (2)	t011 (2)
	CLI-ERY-CIP (1)	Epidemiological (1)	t011 (1)
	CIP-FUS (1)	Clinical (1)	t011 (1)
	CLO-CIP (1)	Clinical (1)	t011 (1)
	MUP-CIP (1)	Clinical (1)	t011 (1)
	TOB-CIP (1)	Clinical (1)	t011 (1)
3	CIP (29)	Clinical (24), Epidemiological (5)	t011 (22), t034 (1), t108 (1), t899 (1), t1197 (1), t1451 (2), t2970 (1)
	GEN-TOB (2)	Epidemiological (2)	t011 (1), t2123 (1)
	MUP (2)	Clinical (1), Epidemiological (1)	t011 (1)
	CLI (1)	Clinical (1)	t011 (1)
TOTAL	GEN (22), TOB (29), CLO (5), CLI (65), ERY (53), MUP (8), CIP (92)	Clinical (87), Epidemiological (21)	t011 (79), t034 (5), t108 (2), t899 (2), t1197 (2), t1255 (2), t1451 (8), 1456 (1), t1939 (3),t2123 (1), t2346 (1), t2970 (1), t18071 (1)

[a]Families considered for MDR phenotype were: β-lactams, aminoglycosides, folate pathway antagonists, glycopeptides, lincosamides, lipopeptides, macrolides, oxazolidinones, phenicols, fluoroquinolones, fusidanes, tetracyclines, mupirocin. All isolates were resistant to both β-lactam and tetracycline families, not considered in the phenotype description but included in the number of different families for the MDR count.

[b]Antimicrobial agents: TET, tetracycline; ERY, erythromycin; CLI, clindamycin; GEN, gentamicin; TOB, tobramycin; CIP, ciprofloxacin; FUS, fusidic acid; MUP, mupirocin; CLO, chloramphenicol.

[c]Number of isolates in parenthesis.