Epidemiology of methicillin-resistant *Staphylococcus aureus* CC398 in hospitals located in Spanish regions with different pig farming densities: a multicentre study

Sara CEBALLOS[1], Carmen ASPIROZ[2], Laura RUIZ-RIPA[1], Esteban REYNAGA[3], José Manuel AZCONA-GUTIÉRREZ[4], Antonio REZUSTA[5], Cristina SERAL[6], Fernando ANTOÑANZAS[7], Luis TORRES[8], Concepción LÓPEZ[9], Lorena LÓPEZ-CERERO[10], Emilia CERCENADO[11], Myriam ZARAZAGA[1], Carmen TORRES[1*] on behalf of the Study Group of clinical LA-MRSA**

Running title: Epidemiology of clinical MRSA CC398 in Spain

[1]Área Bioquímica y Biología Molecular, Universidad de La Rioja, Logroño, Spain;
[2]Servicio Microbiología, Hospital Royo Villanova, Zaragoza, Spain;
[3]Servicio Microbiología, Hospital Universitari de Vic, Vic, Spain;
[4]Servicio Microbiología, Hospital San Pedro, Logroño, Spain;
[5]Servicio Microbiología, Hospital Universitario Miguel Servet/ IIS Aragón, Zaragoza, Spain;
[6]Servicio Microbiología, Hospital Clínico Universitario Lozano Blesa, Zaragoza, Spain;
[7]Departamento de Economía, Universidad de La Rioja, Logroño, Spain
[8]Servicio Microbiología, Hospital San Jorge, Huesca, Spain;
[9]Servicio Microbiología, Hospital de Barbastro, Barbastro, Spain;
[10]Servicio Microbiología, Hospital Virgen Macarena, Sevilla, Spain;
[11]Servicio Microbiología, Hospital Universitario Gregorio Marañón, CIBERES, Centro de Investigación Biomédica en Red de Enfermedades Respiratorias, Madrid, Spain.

***Corresponding author**
Professor Carmen Torres
Área de Bioquímica y Biología Molecular
Facultad de Ciencia y Tecnología; Universidad de La Rioja
Madre de Dios 51, 26006, Logroño, Spain
Tel.: +34 941299750; fax: +34 941299721 ; E-mail: carmen.torres@unirioja.es
** Members are listed in the Acknowledgements section.

Synopsis

Objectives: Tetracycline resistance (Tet^R) is a marker of livestock-associated (LA)-MRSA of lineage CC398. The objective was to determine the MRSA CC398 prevalence among Tet^R-MRSA recovered in Spanish hospitals located in regions with different pig-farming-densities, and the influence of pig density as a key risk factor for its acquisition.

Methods: Tet^R-MRSA isolates (n=232) recovered from clinical and epidemiological samples during January-June 2016 in 20 hospitals of 13 regions with different pig-farming densities were analysed. MRSA CC398 identification, detection of *spa*-types, methicillin resistance genes, and immune evasion cluster (IEC) genes were performed by PCR/sequencing. Statistical analyses were performed to establish the relationships between MRSA CC398 prevalence and pig density.

Results: The global MRSA prevalence was 29.7 % (6.9% Tet^R-MRSA/MRSA), with 137 CC398 recovered isolates, representing 4.1% of total MRSA and 59.1% of Tet^R-MRSA. Among MRSA CC398, 16 different *spa*-types were recorded (t011: 72.3%), and all but two strains were IEC-negative. Higher pig-density regions were associated with significant MRSA CC398 increases in hospitals located in adjacent regions ($p<0.001$). Linear regression models explained the relationships between MRSA CC398 and pig density ($p<0.001$), with a rise of 6.6 MRSA CC398 cases per 100 MRSA when a region increases 100 pigs/km^2

Conclusions: High pig density leads to a significant increase of MRSA CC398 among hospitals in Spain, and the combination with high human population could help to its dissemination. In Spain, the prevalence of the zoonotic CC398 lineage is tightly related

to pig-farming density; therefore specific tools could be implemented in order to detect its dissemination.

Introduction

Staphylococcus aureus is an important opportunistic pathogen, and CC398 is the most common genetic lineage of livestock-associated (LA)-MRSA, firstly discovered in pigs in 2005.[1] Transmission of LA-MRSA CC398 from livestock to humans has been frequently reported, usually as colonization, but more and more often as a cause of infection.[2] Contact with livestock is a well-known risk factor for human LA-MRSA carriage,[3] and studies reflecting the influence of pig density in LA-MRSA CC398 colonization were previously done in some European countries.[4,5]

The vast majority of LA-MRSA CC398 presents tetracycline resistance (Tet^R),[6] probably due to the high use of this antimicrobial in food animals, and Tet^R can be used as a marker for LA-MRSA CC398 detection within clinical or epidemiological MRSA.[2,7]

The immune evasion gene cluster (IEC) is a set of genes contained by the ϕSaint3 prophage that allows *S. aureus* to avoid the first barrier in human immune responses, and it is usually present in strains adapted to humans.[8] The *scn* gene is present in all types of IEC, therefore it is considered a marker gene of this cluster.[9] Few cases of invasive infections in humans by LA-MRSA CC398 containing the IEC system have been described; [10] representing a potential process of re-adaptation to humans. [11]

Previous studies in Spain showed MRSA CC398 prevalence in particular hospitals, but non correlation analysis with pig density have been done before.[2,7,12,13] For the reasons indicated above, the aim of this study was to analyse the real burden of MRSA CC398 in different hospitals from areas with varied pig-farming densities in Spain and to determine if pig density could be a risk factor for LA-MRSA acquisition through statistical analyses.

Methods

Bacterial strains and molecular characterization

During January-June 2016, a total of 11,405 *S. aureus* isolates were recovered from different patients in 20 hospitals in Spain, located in 13 different geographic regions showing variable pig-farming densities (Figure 1a, Table 1), and 3,383 were MRSA (from clinical and epidemiological surveillance samples). The MRSA prevalence of 12 of these 20 hospitals in the study period was previously reported.[14] Hospitals of the north of Spain were highly represented in this study, but also other hospitals of the east, centre and south of Spain (with very high, very low, and medium pig-farming density, respectively), were included.

All Tet^R-MRSA isolates of this collection (n=232) were included in this multicentre study. The *spa*-type of all Tet^R-MRSA strains were determined by PCR and sequencing as previously described.[7] Identification of the CC398 lineage was carried out by specific PCR.[15] The presence of *mecA* methicillin-resistance gene was analysed by PCR.[16] The detection of the *scn* gene was carried out by PCR in all CC398 strains. For the *scn*-positive isolates, all five IEC genes were studied, as well as the absence of *hlb* gene.[2]

Statistical analysis

Spearman correlations between pairs of variables were studied in order to measure possible relationships between them (strong correlation: $|\rho|>0.7$, moderate correlation: $0.3<|\rho|\leq 0.7$, and weak correlation: $|\rho|\leq 0.3$). Simple or multiple linear regression analyses were performed to predict the number of cases of MRSA CC398 (dependent variable) respect to the pig and/or population densities (independent variables). A quadratic regression model in pig density was created to check whether it fitted better.

These statistical analyses were performed using the RStudio program (version 1.1.453) ($p<0.05$ was considered statistically significant).

Results and Discussion

Prevalence of MRSA and Tet^R-MRSA CC398

LA-MRSA CC398 dissemination is an issue of great concern, highly related to pig-farming. In this study, we present the prevalence of this genetic lineage in 20 Spanish hospitals located in 13 regions with different pig-densities. The global MRSA prevalence in the studied hospitals was 29.7%, and within MRSA, 232 strains (6.9%) presented the selected Tet^R phenotype (Table 1). All Tet^R-MRSA were *mecA*-positive.

The CC398 lineage was present in 137 strains out of the 232 Tet^R-MRSA isolates (59.1%), representing 4.1% of total MRSA. This supports the idea of Tet^R as a suitable marker for MRSA CC398 detection, as previously suggested.[2,7] The distribution of MRSA CC398 isolates among the analysed hospitals was heterogeneous (Table 1, Figure 1a), with a higher proportion of MRSA CC398/MRSA in those hospitals located in regions with higher pig densities. On the other hand, hospitals in areas with low pig densities showed lower rates of MRSA CC398/MRSA, and this lineage was totally absent for some hospitals (Table 1).

The hospital H1 located in region R1 (high pig density: 247.5 pigs/km^2) showed a very high prevalence of MRSA CC398 (31% Tet^R-MRSA CC398/MRSA), and more than 80% of Tet^R-MRSA strains belonged to lineage CC398 (Table 1). These findings strongly support those previously described in that hospital, in which 32% of MRSA isolated from 2012 to 2015 were typed as CC398, with 88% of Tet^R-MRSA ascribed to CC398.[12]On the other hand, hospital H14 located in region R9 (low pig density: 18.3 pigs/km^2), presented a low rate (3.6%) of Tet^R-MRSA CC398/MRSA (and 7% Tet^R-

MRSA/MRSA) (Table 1). In a previous study performed in that hospital in two different periods (2001 and 2009),[16] the CC398 lineage was not detected in either of the periods, with a very low prevalence of Tet^R-MRSA (2% in 2001 and 1% in 2009). Given these new data, CC398 can be considered as an emergent human genetic lineage in this area.

According on our data, the LA-MRSA CC398 clone may contribute to increase the global prevalence of MRSA, since the hospital with the highest percentage of Tet^R-MRSA CC398/MRSA (31%) corresponded to the one with the highest MRSA/*S. aureus* prevalence (71%). Moreover, the four hospitals with the highest MRSA prevalence are those with very high frequency of CC398 lineage, suggesting an association.

Sample origin

According to the origin of CC398 isolates (Table S1), 25.5% of them were obtained from epidemiological surveillance. Within the remaining 74.5% recovered from clinical samples, 74% were obtained from skin soft tissue infections (SSTI) and respiratory tract infections (RTI), in accordance with the ways of transmission of this genetic lineage through direct contact or airway.[17] The clonal complex CC398 has been strongly associated with SSTI in other studies.[8] Origins from all Tet^R-MRSA isolates are shown in Table S2.

Molecular characterization by spa-type

Sixteen different *spa*-types were detected among Tet^R-MRSA CC398 isolates (Figure 1b, Table S1) (72.3% t011, 7.3% t1451, 4.4% t034, 2.9% t899, 2.2% t1939 and 10.9% others). t011 is one of the most representative CC398 *spa*-types in pigs[18,19] and also in human MRSA CC398 infections, in Spain and in other countries.[3,7,12,13,20] Nevertheless,

when the infections are caused by MSSA CC398, presumably from human origin, t571 is the prevalent *spa*-type.[19]

The distribution of MRSA CC398 *spa*-types according to hospitals can be seen on Table S3. A new *spa*-type was detected among the MRSA CC398 strains analysed (t18071). On the other hand, 40% of Tet^R-MRSA non-CC398 isolates was typed as t127, which belongs to CC1, a typical community-associated MRSA clonal complex, also described in livestock.[11] This way, Tet^R may be a good marker not only for MRSA CC398 but also for other LA-MRSA genetic lineages, such as CC1/t127.

Presence of IEC system

More than 98% of Tet^R-MRSA C398 lacked the IEC genes, suggesting an animal origin for the analysed strains. However, two MRSA CC398 were *scn*-positive: 1) a t011 strain recovered from RTI (hospital H12) ascribed to IEC type-E (*scn*, *sak*); 2) a t1939 strain obtained from SSTI (hospital H11), ascribed to IEC type-B (*scn*, *chp*, *sak*) (Table S1). The presence of IEC genes is very uncommon among LA-MRSA CC398,[8,10] but it is frequently found in MSSA CC398 isolates related to an ancestral human clade, that, according to some authors could be the origin of LA-MRSA CC398.[8] As expected, these two strains carrying the IEC system lacked the β-hemolysin-encoding gene (*hlb*), which is the preferential integration site of ϕSaint3 phages.[9] We also checked the presence of *hlb* gene in a representative collection of IEC-negative strains with different *spa*-types and from different hospitals, and all isolates were *hlb*-positive. The detection of MRSA CC398 IEC-positive strains, carrying typical markers of LA-MRSA such as tetracycline resistance, is of concern and relevance, and this phenomenon could be part of the re-adaptive process of this animal genetic lineage to humans, and it would be interesting to characterize them in more detail.

Relationships between Tet^R-MRSA CC398 and pig or human population densities

We established statistical models that predicted the presence of Tet^R-MRSA CC398 considering the pig and human population densities (Table S4). We observed a strong and statistically significant association between the rate of MRSA CC398/MRSA (or MRSA CC398/ *S. aureus*) of the hospital, and the pig density of the adjoining region ($p<0.05$). However, MRSA CC398/MRSA is not associated to human population density by its own ($p>0.05$). We observed that the higher number of pigs/km^2 in a region, the greater number of MRSA CC398/MRSA (or MRSA CC398/*S. aureus*) detected in hospitals located in those regions.

Moreover, the simple linear regression models showed that MRSA CC398/MRSA rate increases with the pig density ($p<0.001$), but not when only the density of human population is the predictor variable ($p>0.05$) (Table S4). The models created stated that an increase of 100 pigs/km^2 in a region involves a rise of 6.6 cases of Tet^R-MRSA CC398 per 100 MRSA cases or 4 cases per 100 *S. aureus* (Table S4). If a multiple linear regression model with both, pig and human population densities, is established, the R^2 adjusted by degrees of freedom improves, hence, becoming this one another valid model (Table S4). The quadratic regression analysis performed showed a slight decrease in R^2 (0.635), so it was discarded. These findings point to pigs as the key factor in dissemination, although high rates of human population could also play a role and contribute to subsequent dissemination in the context of high pig density (as the multiple linear regression model shows). Previous studies reported the detection of Tet^R-MRSA CC398 clinical isolates in patients without contact with farm animals, suggesting that human to human transmission may occur, mainly in areas of high farming density.[2]

Conclusions

In conclusion, pig density leads to an increase of MRSA CC398 cases among hospitals, and this, in combination with a high human population might help to the dissemination of this lineage. The new statistical regression models allow the prediction of the rate of CC398 infections in an area by knowing its pig density. The rapid detection of MRSA CC398 in hospitals, particularly those located in high-pig-farming-density areas, is a need due to their increasing implication in human infections and the possibility of dissemination in this setting.

Acknowledgments

Part of this work was presented at the 28th ECCMID, Madrid, Spain (poster P1727) and at the 29th ECCMID, Amsterdam, The Netherlands (P2548).

Members of the Study Group of clinical LA-MRSA

José Leiva (Clínica Universidad de Navarra, Pamplona); Carmen Ezpeleta, Carmen Martín (Complejo Hospitalario de Navarra, Pamplona); Javier Castillo (Hospital Clínico Universitario Lozano Blesa, Zaragoza); Jorge Arribas, Carmen Navarro (Hospital de Alcañiz, Alcañiz, Teruel); Ana Betrán (Hospital de Barbastro, Barbastro, Huesca); Jose Luis Hernández (Hospital de Cruces, Bilbao); Pilar Berdonces (Hospital de Galdakao, Galdakao, Vizcaya); Inés de Benito, Ana Belén Campo (Hospital de Sierrallana, Torrelavega, Cantabria); Antonina Arias, Blanca Fortuño (Hospital Ernest Lluch Martin, Calatayud, Zaragoza); Javier Pereira (Hospital Royo-Villanova, Zaragoza); Ana Milagro (Hospital San Jorge, Huesca); Luis Miguel Soria-Blanco, Carla Andrea Alonso (Hospital San Pedro, Logroño); Carmen Gimeno, Belén Ulibarri (Hospital Santiago Apóstol, Miranda de Ebro, Burgos); Raquel Calleja, Mercé García (Hospital Universitari Arnau de Vilanova, Lérida); María Navarro, Anna Vilamala (Hospital

Universitari de Vic, Vic, Barcelona); Andrés Canut, Mª Luz Cordón (Hospital Universitario de Álava, Vitoria); Ledicia Álvarez, Gregoria Megías (Hospital Universitario de Burgos, Burgos); María Gomáriz (Hospital Universitario de Donostia, San Sebastián); Jorge Calvo, Marina Fernández (Hospital Universitario Marqués de Valdecilla, Santander); Ana Isabel López-Calleja (Hospital Universitario Miguel Servet/ IIS Aragón, Zaragoza).

Funding

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

Transparency section

None to declare.

References

1. Voss A, Loeffen F, Bakker J, Klaassen C, Wulf M. Methicillin-resistant *Staphylococcus aureus* in pig farming. *Emerg Infect Dis* 2005; **11**: 1965–6.

2. Benito D, Lozano C, Rezusta A, *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.

3. Van Den Broek IVF, Van Cleef BAGL, Haenen A, *et al.* Methicillin-resistant *Staphylococcus aureus* in people living and working in pig farms. *Epidemiol Infect* 2009; **137**: 700.

4. Sieber RN, Skov RL, Nielsen J, *et al.* Drivers and dynamics of methicillin-resistant livestock-associated *Staphylococcus aureus* CC398 in pigs and humans in Denmark. *MBio* 2018; **9**: 1–12.

5. Feingold BJ, Silbergeld EK, Curriero FC, van Cleef BAGL, Heck MEOC, Kluytmans JAJW. Livestock density as risk factor for livestock-associated methicillin-resistant *Staphylococcus aureus*, the Netherlands. *Emerg Infect Dis* 2012; **18**: 1841–9.

6. Price LB, Stegger M, Hasman H, *et al. Staphylococcus aureus* CC398: Host adaptation and emergence of methicillin resistance in livestock lance. *MBio* 2012; **3**: 1–6.

7. Lozano C, Rezusta A, Gómez P, *et al.* High prevalence of *spa* types associated with the clonal lineage CC398 among tetracycline-resistant methicillin-resistant *Staphylococcus aureus* strains in a Spanish hospital. *J Antimicrob Chemother* 2012; **67**: 330–4.

263 8. Cuny C, Wieler L, Witte W. Livestock-associated MRSA: The impact on humans.
264 *Antibiotics* 2015; **4**: 521–43.

265 9. Jung P, Abdelbary MMH, Kraushaar B, *et al.* Impact of bacteriophage *Saint*3
266 carriage on the immune evasion capacity and hemolytic potential of *Staphylococcus*
267 *aureus* CC398. *Vet Microbiol* 2017; **200**: 46–51.

268 10. Pérez-Moreno MO, Centelles-Serrano MJ, Nogales-López J, Domenech-Spanedda
269 MF, Lozano C, Torres C. Unusual presence of the immune evasion gene cluster in
270 livestock-associated MRSA of lineage CC398 causing peridural and psoas abscesses in
271 a poultry farmer. *Enferm Infecc Microbiol Clin* 2017; **35**: 651-4.

272 11. Zarazaga M, Gómez P, Ceballos S, Torres C. Molecular epidemiology of
273 *Staphylococcus aureus* lineages in the animal–human interface. In: *Staphylococcus*
274 *aureus*. Academic Press (Elsevier Inc), 2017; 189–214.

275 12. Reynaga E, Torres C, Garcia-Nuñez M, *et al.* Clinical impact and prevalence of
276 MRSA CC398 and differences between MRSA-TetR and MRSA-TetS in an area of
277 Spain with a high density of pig farming: a prospective cohort study. *Clin Microbiol*
278 *Infect* 2017; **23**: 678.e1-678.e4.

279 13. Camoez M, Sierra JM, Pujol M, Hornero A, Martin R, Domínguez MA. Prevalence
280 and molecular characterization of methicillin-resistant *Staphylococcus aureus* ST398
281 resistant to tetracycline at a Spanish hospital over 12 years. *PLoS One* 2013; **8**: e72828.

282 14. Ceballos S, Aspiroz C, Ruiz-Ripa L, *et al.* Multicenter study of clinical non-β-
283 lactam-susceptible MRSA strains: genetic lineages and PVL production. *Enferm Infecc*
284 *Microbiol Clin* 2019; pii: S0213-005X(19)30132-6.

285 15. Stegger M, Lindsay JA, Moodley A, Skov R, Broens EM, Guardabassi L. Rapid

286 PCR detection of *Staphylococcus aureus* clonal complex 398 by targeting the
287 restriction-modification system carrying *sau1-hsdS1*. *J Clin Microbiol* 2011; **49**: 732–4.

288 16. Lozano C, Porres-Osante N, Crettaz J, *et al.* Changes in genetic lineages, resistance,
289 and virulence in clinical methicillin-resistant *Staphylococcus aureus* in a Spanish
290 hospital. *J Infect Chemother* 2013; **19**: 233–42.

291 17. Smith TC. Livestock-Associated *Staphylococcus aureus*: The United States
292 experience. *PLoS Pathog* 2015; **11**: 1–9.

293 18. Rasschaert G, Vanderhaeghen W, Dewaele I, *et al.* Comparison of fingerprinting
294 methods for typing methicillin-resistant *Staphylococcus aureus* sequence type 398. *J*
295 *Clin Microbiol* 2009; **47**: 3313–22.

296 19. Uhlemann A-C, Porcella SF, Trivedi S, *et al.* Identification of a Highly
297 Transmissible animal-independent *Staphylococcus aureus* ST398 clone with distinct
298 genomic and cell adhesion properties. *MBio* 2012; **3**: 1–9.

299 20. Nielsen RT, Kemp M, Holm A, *et al.* Fatal septicemia linked to transmission of
300 MRSA clonal complex 398 in hospital and nursing home, Denmark. *Emerg Infect Dis*
301 2016; **22**: 900–2.

302

Table 1. Distribution of isolates, prevalence of MRSA and prevalence of Tet^R-MRSA CC398 according to the analysed hospitals. Pig and human population densities are shown per region.

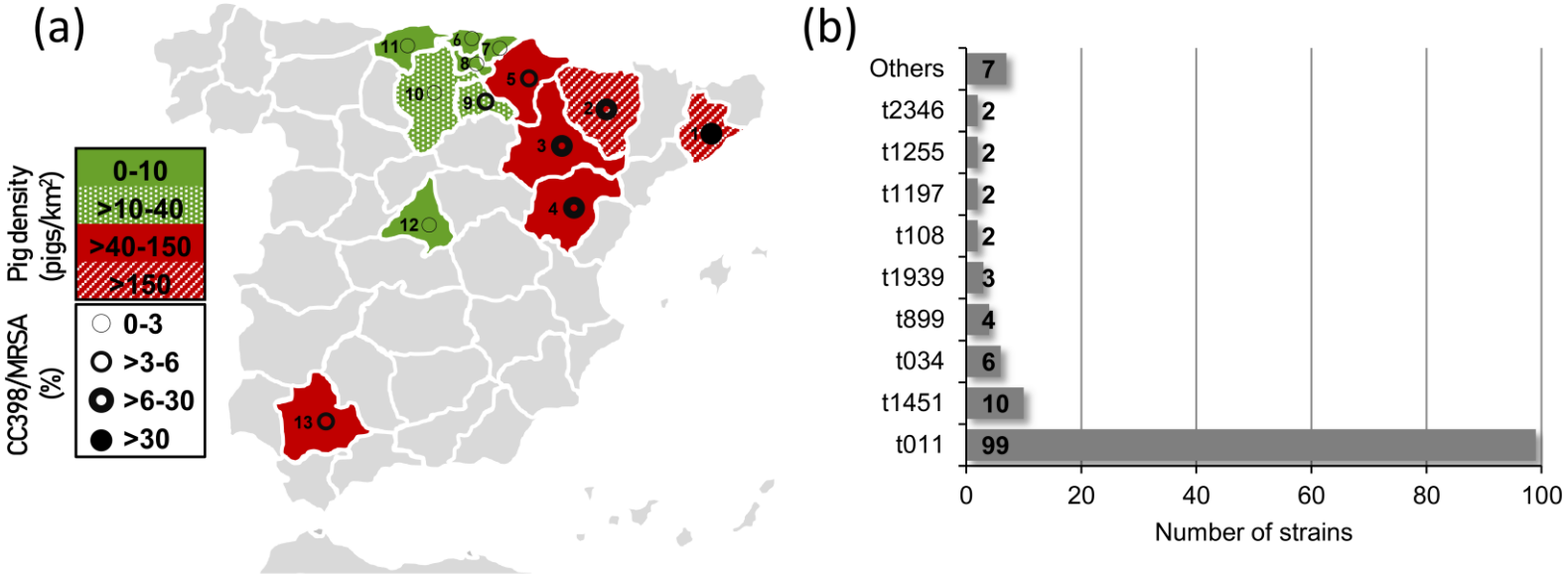
		Number of strains				Rates (%)			Density[c] per region	
Hospital number[a]	Region number[b]	*S. aureus*	MRSA	Tet^R-MRSA	Tet^R-MRSA CC398	MRSA/ *S. aureus*	Tet^R-MRSA CC398/ MRSA	Tet^R-MRSA CC398/ Tet^R-MRSA	Pigs/km^2	Habitants/km^2
H1	R1	122	87	33	27	71.3%	31%	81.8%	247.46	717.36
H2	R2	341	135	20	19	39.6%	14.1%	95%	217.68	14.05
H3	R2	575	328	24	15	57.0%	4.6%	62.5%	217.68	14.05
H4	R3	1024	251	34	18	24.5%	7.2%	52.9%	142.66	55.20
H5	R3	670	175	20	9	26.1%	5.1%	45%	142.66	55.20
H6	R3	180	76	9	7	42.2%	9.2%	77.8%	142.66	55.20
H7	R3	126	42	6	3	33.3%	7.1%	50%	142.66	55.20
H8	R4	99	36	4	4	36.4%	11.1%	100%	69.97	9.15
H9	R5	304	36	2	2	11.8%	5.6%	100%	50.89	61.90
H10	R5	799	206	14	7	25.8%	3.4%	50%	50.89	61.90
H11	R13	250	84	7	3	33.6%	3.6%	42.9%	42.10	138.18
H12	R10	666	220	6	3	33%	1.4%	50.0%	27.66	25.54
H13	R10	113	42	2	0	37.2%	0%	0%	27.66	25.54
H14	R9	368	112	6	4	30.4%	3.6%	66.7%	18.31	62.51
H15	R8	978	334	5	5	34.2%	1.5%	100%	4.99	107.53
H16	R7	1009	130	7	3	12.9%	2.3%	42.9%	3.56	360.18
H17	R12	1480	315	12	0	21.3%	0%	0%	2.85	810.66
H18	R6	762	277	7	3	36.4%	1.1%	42.9%	2.19	517.95
H19	R11	1124	371	13	5	33.0%	1.3%	38.5%	0.45	109.06
H20	R11	415	126	1	0	30.4%	0%	0%	0.45	109.06
		11405	3383	232	137	29.7%	4.1%	59.1%		

[a]Numeric code used for the analysed hospitals (H). H1: H. Universitari de Vic; H2: H. de Barbastro; H3: H. San Jorge; H4: H. Universitario Miguel Servet; H5: H. Universitario Lozano Blesa; H6: H. Royo Villanova; H7: H. Ernest Lluch Martin; H8: H. de Alcañiz; H9: Clínica Universitaria de Navarra; H10: Complejo Hospitalario de Navarra; H11: H. Virgen Macarena; H12: H. Universitario de Burgos; H13: H. Santiago Apóstol; H14: H. San Pedro; H15: H. Universitario de Álava; H16: H. Universitario de Donostia; H17: H. Universitario Gregorio Marañón; H18: H. de Galdakao; H19: H. Marqués de Valdecilla; H20: H. Sierrallana.

[b]Numeric code used for the analysed regions (R). R1: Barcelona; R2: Huesca; R3: Zaragoza; R4: Teruel; R5: Navarra; R6: Bizkaia; R7: Gipuzkoa; R8: Álava; R9: La Rioja; R10: Burgos; R11: Cantabria; R12: Madrid; R13: Sevilla.

[c]Surface area data (km2) and number of inhabitants per region were obtained from the National Statistics Institute of Spain (2017). Number of pigs per region was obtained from the annual pig report (2015) edited by the Ministry of Agriculture and Fisheries, Food and Environment of Spain.

Figure 1. **(a)** Map of Spain with the location of the analysed hospitals (hospitals H1-H20) representing pig densities (colours) and the presence of Tet^{R}-MRSA CC398 (circles) in the 13 studied regions (numbers). R1: Barcelona (H1); R2: Huesca (H2, H3); R3: Zaragoza (H4-H7); R4: Teruel (H8); R5: Navarra (H9, H10); R6: Bizkaia (H18); R7: Gipuzkoa (H16); R8: Álava (H15); R9: La Rioja (H14); R10: Burgos (H12, H13); R11: Cantabria (H19, H20); R12: Madrid (H17); R13: Sevilla (H11). **(b)** Main *spa*-types detected among the Tet^{R}-MRSA CC398 strains. The category "others" belongs to those *spa*-types with only one strain (t1456, t2123, t2370, t2383, t2741, t2970 and t18071).



Supplementary data

Table S1. Molecular typing and genotypic characterization of the 137 Tet^{R}-MRSA CC398 strains.

spa type	No of strains	Sample origin (No of strains)	IEC (No of strains)
t011	99	SSTI[b] (35), ES[c] (25), RTI[d] (17), SSI[e] (14), UTI[f] (5), blood (3)	E[g] (1)
t1451	10	SSTI (4), RTI (3), SSI (1), ES (1), UTI (1)	-
t034	6	SSTI (2), RTI (2), ES (1), blood (1)	-
t899	4	SSTI (2), ES (2)	-
t1939	3	SSTI	B[g] (1)
t108	2	SSTI (1), ES (1)	-
t1197	2	ES	-
t1255	2	SSTI (2)	-
t2346	2	SSTI (1), ES (1)	-
t1456	1	RTI	-
t2123	1	ES	-
t2370	1	RTI	-
t2383	1	SSTI	-
t2741	1	ES	-
t2970	1	blood	-
t18071[a]	1	SSTI	-

[a]New *spa* type; [b]SSTI: skin and soft tissue infections; [c]ES: epidemiological surveillance; [d]RTI: respiratory tract infections; [e]SSI: surgical site infections; [f]UTI: urinary tract infection; [g]IEC type E containing *scn* adn *sak* genes, IEC type B containing *scn*, *chp* and *sak* gene.

Table S2. Sample origins of all Tet^{R}-MRSA isolates recovered in this study.

Origin	MRSA CC398	MRSA no-CC398	TOTAL
SSTI[a]	52	49	101
ES[b]	35	19	54
RTI[c]	24	14	38
SSI[d]	15	4	19
UTI[e]	6	5	11
Blood	5	4	9
TOTAL	137	95	232

[a]SSTI: skin and soft tissue infections; [b]ES: epidemiological surveillance; [c]RTI: respiratory tract infections; [d]SSI: surgical site infections; [e]UTI: urinary tract infection.

Table S3. Different *spa* types detected among Tet^{R}-MRSA CC398 isolates according to hospitals.

Hospital number	*spa* types (No of strains)
H1	t011 (19), t034 (2), t108 (2), t1197 (1), t1451 (1), t2370 (1), t18071[a] (1)
H2	t011 (14), t034 (1), t1451 (3), t899 (1)
H3	t011 (12), t034 (1), t1451 (2)
H4	t011 (15), t034 (1), t1451 (1), t2741 (1)
H5	t011 (7), t2383 (1), t2970 (1)
H6	t011 (5), t1197 (1), t1456 (1)
H7	t011 (3)
H8	t011 (2), t1451 (1), t2346 (1)
H9	t011 (2)
H10	t011 (5), t1451 (1), t2123 (1)
H11	t011 (2), t1451 (1), t2346 (1)
H12	t011 (2), t899 (1)
H13	-
H14	t011 (2), t1939 (1)
H15	t011 (3), t1939 (2)
H16	t011 (1), t034 (1), t899 (1)
H17	-
H18	t011 (1), t1255 (2)
H19	t011 (4), t899 (1)
H20	-

[a]New *spa* type.

Table S4. Correlation and linear regression results between Tet^{R}-MRSA CC398 cases and pig or human population densities.

	Spearman correlations	Linear regression models	
Variables	rho (p)	R^2 (F value; p)	β (t value; p)
MRSA CC398/ MRSA[a] pigs/ km^2 [b]	ρ=0.86 (p<0.001)	R^2=0.58 (F=25.21; p<0.001)	β_0=5.5853e-03 (t=0.38; p=0.71) β_1=6.553e-04 (t=5.02; p<0.001)
MRSA CC398/ MRSA[a] inhabitants/ km^2 [b]	ρ=-0.39 (p=0.09)	R^2=0.06 (F=1.23; p=0.28)	β_0=4.387e-02 (t=2.25; p=0.03) β_1=7.535e-05 (t=1.11; p=0.28)
MRSA CC398/ *S. aureus*[a] pigs/ km^2 [b]	ρ=0.89 (p=<0.001)	R^2=0.46 (F=15.22; p=0.001)	β_0=-6.0893e-03 (t=-0.53; p=0.60) β_1=3.978e-04 (t=3.90; p=0.001)
MRSA CC398/ *S. aureus*[a] inhabitants/ km^2 [b]	ρ=-0.45 (p=0.05)	R^2=0.16 (F=3.51; p=0.08)	β_0=1.100e-02 (t=0.87; p=0.39) β_1=8.233e-05 (t=1.87; p=0.08)
MRSA CC398/ MRSA[a] pigs/ km^2 [b] inhabitants/ km^2 [b]		R^2=0.68 (F=17.86; p<0.001) R^2 adjusted=0.64	β_0=-1.122e-02 (t=-0.74; p=0.47) β_1=6.735e-04 (t=5.69; p<0.001) β_2=9.146e-05 (t=2.23; p=0.04)
MRSA CC398/ *S. aureus*[a] pigs/ km^2 [b] inhabitants/ km^2 [b]		R^2=0. 66(F=16.67; p<0.001) R^2 adjusted=0.62	β_0=-2.305e-02 (t=-2.16; p=0.05) β_1=4.162e-04 (t=5.01; p<0.001) β_2=9.229e-05 (t=3.21; p=0.01)

[a]Dependent variable used in the regression models; [b]Independent variable used in the regression mod