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Penicillin susceptibility among invasive MSSA infections: a multicentre study in 16 Spanish hospitals

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Objectives: To determine the prevalence of penicillin susceptibility among MSSA causing bloodstream infections (BSIs) in 16 Spanish hospitals and to characterize the penicillin-susceptible MSSA (MSSA-PEN^S) isolates.

Methods: A total of 1011 *Staphylococcus aureus* isolates were collected from blood cultures in 16 Spanish hospitals during 2018–19 (6–12 months) and their susceptibility to 18 antimicrobials was determined. The MSSA-PEN^S isolates were selected and examined by PCR to determine the presence of the *blaZ* gene, other resistance genes and the genes *lukF/lukS-PV, eta, etb* and *tst*. The immune evasion cluster (IEC) type was also analysed. All the MSSA-PEN^S isolates were submitted to *S. aureus* protein A (*spa*) typing and the clonal complexes (CCs) were assigned according to their *spa* type.

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Results: The prevalence of MSSA was 74.6% (754/1011) and 14.9% (151/1011) were MSSA-PEN^S-*blaZ*^{negative}. MSSA-PEN^S-*blaZ*^{negative} isolates ($n = 151$) were ascribed to 88 *spa* types and 11 CCs. The most frequent CCs were CC5 (35/151) and CC398 (25/151), with t002-CC5 and t571-CC398 being the most common lineages. Pan-susceptibility was identified in 117 of the 151 MSSA-PEN^S-*blaZ*^{negative} isolates (77.5%). In the remaining isolates, erythromycin and clindamycin resistance was the most frequent resistance found, although tobramycin, ciprofloxacin, fusidic acid, mupirocin and/or tetracycline resistance was also detected. Thirty-eight MSSA-PEN^S-*blaZ*^{negative} isolates were IEC negative and four isolates were Panton–Valentine leucocidin ('PVL') positive.

Conclusions: A high penicillin susceptibility rate was detected among MSSA, opening therapeutic opportunities for BSIs. The emergence of new successful MSSA-PEN^S clones could be responsible for these data. The detection among MSSA-PEN^S-*blaZ*^{negative} isolates of the clonal lineage CC398 or the absence of an IEC raises questions about their possible animal origin, requiring further analysis.

Introduction

Staphylococcus aureus is an opportunistic pathogen associated with different diseases, from skin and soft tissue infections to severe bacteraemia, endocarditis and pneumonia. Bloodstream infections (BSIs) caused by *S. aureus* are associated with high morbidity and mortality.[1] Among the antimicrobial resistance of *S. aureus*, methicillin resistance is highly relevant since it provides resistance to almost all β-lactam agents, seriously limiting therapeutic options. In many studies, MRSA BSIs have been associated with worse clinical outcomes than those caused by MSSA,[2,3]

although other studies failed to find significant differences between these types of infections.[1] Nonetheless, survival of patients with *S. aureus* BSIs can be improved with early infectious disease consultations and optimal care processes with the use of appropriate antibiotics.[4]

The proportion of *S. aureus* BSIs due to MSSA, and more specifically those susceptible to penicillin (MSSA-PEN^S), although variable in the different studies (15%–45%), seems to be gradually increasing; reported in some countries in Europe, the USA, Canada and Australia.[5–11] However, surveillance of MSSA-PEN^S isolates is often not undertaken or reported, as hospital microbiologists and clinicians are accustomed to historically high rates of penicillin resistance. Penicillin is an excellent antimicrobial that offers pharmacokinetic advantages over other β-lactams and potentially could improve clinical outcomes.[5] Moreover, penicillin has a very narrow spectrum of action and is therefore less likely to contribute to the emergence and spread of antimicrobial resistance. Hence penicillin might be a useful addition to other treatment options and first-choice antibiotics for MSSA BSIs, such as cefazolin and cloxacillin.[4,12]

A previous study of MSSA-PEN^S undertaken in a small hospital in Spain by our group, found that 22.2% of MSSA BSI isolates were susceptible to penicillin (*blaZ* negative), representing 14.3% of total *S. aureus*.[13] Different clonal lineages were identified among the MSSA-PEN^S-*blaZ*^{negative} isolates of this study,[13] including one CC398 strain (where CC stands for clonal complex). The CC398 lineage has been strongly associated with livestock (especially pigs) in the case of MRSA isolates,[14] although this relationship is not clear in the case of CC398 MSSA isolates.[15] To provide more robust data that give a better national picture of the current situation of MSSA-PEN^S in our country, we report here the findings of a multicentre study involving 16 hospitals. The aim of the present study was to determine the prevalence of penicillin susceptibility among MSSA isolates causing bacteraemia in 16 Spanish hospitals, as well as to characterize the obtained isolates.

Materials and methods

Bacterial isolates

A total of 1011 *S. aureus* isolates (1 isolate/patient) were collected from blood cultures in 16 Spanish hospitals during 2018–19 (12 hospitals, 12 months; and 4 hospitals, 6–9 months) (Table S1, available as Supplementary data at *JAC* Online).[15] Staphylococci were identified to species level based on colony morphology, Gram staining, catalase production, coagulase testing (i.e. Slidex Staph Plus; bioMérieux, Marcy-l'Étoile, France), MALDI-TOF MS, the Vitek2 System (GP card; bioMérieux) or the MicroScan WalkAway System (Beckman Coulter), depending on each hospital's procedures. Resistance to amikacin, cefoxitin, chloramphenicol, ciprofloxacin, clindamycin, daptomycin, erythromycin, fosfomycin, fusidic acid, gentamicin, linezolid, mupirocin, penicillin, teicoplanin, tetracycline, tobramycin, trimethoprim/sulfamethoxazole and vancomycin was assessed using automatic methods (Table S1). Breakpoints were considered according to EUCAST and/or CLSI, depending on each hospital. MSSA-PEN^S isolates of this collection (156 isolates) were included in the present multicentre study.

Detection of antimicrobial resistance genes among MSSA-PEN^S isolates

The presence of the *blaZ* gene in MSSA-PEN^S isolates was studied by PCR.[16] The detection of other non-β-lactam antimicrobial resistance genes [*erm*(A), *erm*(B), *erm*(C), *erm*(T), *msr*(A), *mph*(C), *lnu*(A), *vga*(A), *vga*(C), *ant(4′)-Ia* and *mupA*] according to the antimicrobial resistance profile detected among the isolates was also performed by PCR.[14] Positive and negative controls of the University of La Rioja were used in all PCRs. For further characterization, only MSSA-PEN^S isolates that were negative for the *blaZ* gene were included in this study (MSSA-PEN^S-*blaZ*^{negative}).

Nevertheless, MSSA-PEN^S isolates that carried the *blaZ* gene were re-tested to determine penicillin susceptibility by the following methods: (i) MIC determination by an automatic method (MicroScan WalkAway); (ii) Etest; (iii) disc diffusion (1 U disc); and (iv) nitrocefin determination. Moreover, the mutations in the *blaZ, blaI* and *blaRI* genes of these MSSA-PEN^S-*blaZ*^{positive} isolates were analysed by PCR and sequencing,[16–19] using the sequence of *S. aureus* ATCC 29213 as a reference. The existence of possible revertants was analysed by plating cultures onto Mueller–Hinton agar ('MHA') containing 4× to 128× MIC of penicillin and incubating for 48 h at 37°C, as previously described, and the frequency of revertants was calculated in relation to inoculum size.[20] The type of *blaZ* gene (A–D) was based on the amino acids at positions 119 and 207 (based on the numbering that includes the signal peptide) and amino acid changes at other positions were also analysed to detect different allotypes.[17]

Molecular typing of MSSA-PEN^S isolates

Single-locus DNA sequencing of *S. aureus* protein A (*spa*) was carried out following standard methodology[14] and the obtained sequences were analysed by Ridom Staph-Type software version 1.5.21 (Ridom) for all MSSA-PEN^S isolates. MLST (https://pubmlst.org/organisms/staphylococcus-aureus) was performed for some selected isolates. The presumptive CCs associated with MLST of the isolates were assigned according to the *spa* type detected.[14]

Detection of virulence genes among MSSA-PEN^S isolates

The presence of the genes encoding Panton–Valentine leucocidin (PVL; *lukF/lukS-PV*), exfoliative toxin A and toxin B (*eta* and *etb*) and toxic shock syndrome toxin (*tst*) was determined using PCR.[14] The presence of an immune evasion cluster (IEC) was analysed in order to know the possible animal or human origin of our isolates.[14] For *scn*-positive isolates, all five genes of the IEC system (*scn, chp, sak, sea* and *sep*) were studied to determine the IEC type.[14]

Characterization of CC398 isolates

The data related to the 26 CC398 MSSA isolates (PCR-CC398 lineage detection, antimicrobial resistance phenotype and genotype, virulence content and IEC system) were obtained in a previous study[15] and the data were incorporated into this study.

Results

Prevalence of methicillin and penicillin susceptibility

The number of isolates and the rates of *S. aureus*, MSSA, MSSA-PEN^S and MSSA-PEN^S-*blaZ*^{negative} isolates detected in blood cultures from the 16 Spanish hospitals are shown in Table S1 and Figure S1. The global prevalence of MSSA detected among the total *S. aureus* isolates from blood cultures identified in the 16 Spanish hospitals was 74.6% (754/1011). Among the 754 MSSA isolates, 156 isolates were MSSA-PEN^S and 151 were MSSA-PEN^S-*blaZ*^{negative} (representing 20.6% of MSSA and 15.3% of total *S. aureus*). Most of the isolates (97.4%), reported as penicillin susceptible by routine methodology used in the different hospitals, were *blaZ*^{negative} (Table S1). Only five isolates (initially identified as susceptible to penicillin) were *blaZ*^{positive} by PCR.

Molecular characterization of MSSA-PEN^S-blaZ^{negative} isolates

Eighty-eight different *spa* types were detected among the 151 MSSA-PEN^S-*blaZ*^{negative} isolates and were ascribed to 11 CCs (Table 1). The most common *spa* types detected were t002 (associated with CC5) (11.9%) and t571 (associated with CC398) (8.6%). Five new *spa* types were identified (Table 1). The most frequent CCs detected were CC5 (23.2%), CC398 (16.6%), CC45 (15.9%), CC8 (8.6%) and CC97 (5.3%) (Table 1).

Resistance phenotype and genotype of MSSA-PEN^S-blaZ^{negative} isolates

Pan-susceptibility (susceptibility to all 18 antimicrobials tested) was identified in most of the isolates (77.5%, 117/151). In the remaining isolates, resistance to erythromycin (17.9%, 27/151), clindamycin (inducible phenotype) (17.2%, 26/151), tobramycin (2.6%, 4/151), ciprofloxacin (2%, 3/151), fusidic acid (0.7%, 1/151), mupirocin (0.7%, 1/151) and/or tetracycline (0.7%, 1/151) was detected. Only two isolates were MDR (resistant to at least three antimicrobial families) (Table 1). The resistance genes detected were *erm*(A), *erm*(C), *erm*(T), *msr*(A), *lnu*(A), *vga*(A), *ant(4)-Ia*, *tet*(K) and/or *mup*(A) (Table 1). Nineteen CC398 isolates had the inducible macrolide-lincosamide-streptogramin B resistance phenotype ('MLS$_B$'), mediated by the *erm*(T) gene. Non-β-lactam resistance was found in isolates belonging to CC5, CC6, CC8, CC9, CC45 and CC398 lineages (Figure 1), although most of the resistant isolates were CC398 (Table 1 and Figure 1).

Presence of the IEC system and virulence genes in MSSA-PEN^S-blaZ^{negative} isolates

Of the isolates, 74.8% (113/151) contained the *scn* gene, a marker of the IEC system, and the following types of the IEC system were detected after analysing the five genes of the system (number of isolates): A (1), B (47), C (16), D (8), E (21), F (17) and G (3) (Figure 2). Few virulence genes were detected and four isolates associated with CC5, CC8, CC45 and CC97 lineages were PVL positive (Table 1), all of them being IEC positive. Moreover, three CC398 isolates contained the *eta* gene.

Relationship between the presence of an IEC, resistance phenotype and clonal lineages

All isolates belonging to clonal lineages CC9, CC59 and CC101 were IEC negative, while CC10, CC22, CC30, CC75, CC121 and CC152 isolates were IEC positive (Table 1 and Figure 2). In the remaining clonal lineages, both IEC-positive and -negative isolates were identified. Most CC398 isolates were IEC positive (Figure 2). Some resistance phenotypes, such as tobramycin resistance, tetracycline resistance and fusidic resistance, were only identified in IEC-negative isolates. Among IEC-positive isolates, resistance was detected in isolates presenting IEC types B, C and F (Table 1 and Figure 3). Only 34 antimicrobial-resistant isolates were detected and these isolates were (number of resistant isolates/number of IEC-negative isolates) as follows: CC398 (20/1), CC5 (6/3), CC45 (3/1), CC6 (1/1), CC8 (1/1), CC9 (1/1) and unknown CC (2/1) (Figure 3). Resistance among MSSA-PEN^S-*blaZ*^{negative} isolates was highly associated with CC398 and, in some cases, with IEC-negative isolates (Figure 3).

Characteristics of MSSA-PEN^S- blaZ^{positive} isolates

Five MSSA-PEN^S isolates were *blaZ*^{positive}. The characteristics of these isolates are shown in Table 2. Repetition of penicillin susceptibility determination (by Etest, disc diffusion and nitrocefin) verified that they were phenotypically susceptible. Moreover, one, one and three of these isolates showed types A, B and C of the *blaZ* gene, respectively; some additional amino acid changes were identified among these isolates (Table 2). The sequences of the *blaI* gene were also analysed for these five isolates and they did not include changes, except for the X933 isolate (D21G). The X933 isolate did not exhibit amino acid changes in the deduced sequence of BlaR1, whereas the X973 isolate showed 34 amino acid changes compared with the corresponding sequence of *S. aureus* ATCC 29213 (K157R, T166S, Q168H, S185L, V190I, I191N, R194K, N218H, V223A, H231Q, I237M, N256Y, T260S, S264A, N271K, I272T, P273S, P311L, L320F, L321I, I322M, V323I, I334L, N345S, H347Y, M397L, K417N, E422D, E427D, M434I, I449L, P450S, A455S and A456D). The *blaR1* gene could not be amplified in the remaining three isolates. Moreover, revertants were detected in two of the isolates (X1953 and X973) with a reversion frequency of 1.05×10^{-6} and 4.23×10^{-8}, respectively.

Discussion

The proportion of MRSA and MSSA isolates detected among total *S. aureus* from blood cultures in the 16 Spanish hospitals was 25.4% and 74.6%, respectively. The percentage of MRSA identified in invasive infections is in accordance with the data obtained in Spain in 2018 (24.2%) and 2019 (19.2%) and reported by the ECDC.[21] Fortunately, in recent years, many European countries have developed and implemented national recommendations and guidance documents for preventing the spread of MRSA and decreasing trends have been noted in most of them.[21]

The overall prevalence of MSSA-PEN^S-*blaZ*^{negative} detected in the present multicentre study of MSSA isolates was 20%, which is similar to the prevalence obtained in a previous monocentre Spanish study (22%).[13] In other countries, rates around 15%–45% have been identified ,[5,7–9,22] with increases in the proportion of penicillin-susceptible *S. aureus* reported from the USA, Canada, Finland and France.[7,8,22,23] However, although less common, a decreasing trend in penicillin susceptibility has been reported from Sweden.[9] A possible explanation is the policies of antimicrobial use of the different countries. In Sweden, the use of narrow-spectrum agents has been prioritized, while in the other countries penicillin is not commonly used, particularly for invasive infections.[9,13]

Due to the general increase in penicillin susceptibility observed in recent years, this antimicrobial is being suggested as a possible treatment for MSSA-PEN^S BSIs.[5,7] This is not the first time that an antibiotic has been repurposed and it is of potential interest in the fight against growing antimicrobial resistance.[23] Moreover, the study of penicillin susceptibility would not only allow us to reuse this antibiotic but it would even improve the use of other first-line options. Cefazolin has become a prominent therapy for MSSA infections, but there is concern about the cefazolin inoculum effect (CzIE), a phenomenon mediated by staphylococcal

Table 1. Characteristics of the 151 MSSA-PEN^{S}-*blaZ*^{negative} isolates obtained from blood cultures from 16 hospitals in Spain

CC	*spa* types (no. of isolates)	Resistance phenotype (no. of isolates)	Resistance genotype (no. of isolates)	Virulence genes (no. of isolates)	IEC[e]	
					presence	absence
CC5 (35)	t002 (18)	susceptible (14)	–	*eta* (1)	11	3
		ERY, CLI[a] (2)	*erm*(A) (2)	-	2	–
		TOB (1)	*ant(4)-Ia* (1)	-	–	1
		CIP (1)	-	-	–	1
	t306 (3)	susceptible (3)	–	*eta* (1)	2	1
	t304 (3)	susceptible (3)	-	*lukF/lukS-PV* (1)	3	–
	t855 (1)	ERY, CLI[a] (1)	*erm*(A), *erm*(C) (1)	-	–	1
	t1094 (1)	ERY, CLI[a] (1)	*erm*(A), *msr*(A) (1)	-	1	–
	t045 (3), t067 (1), t601 (1), t954 (1), t985 (1), t1062 (1), t2915 (1)	susceptible (9)	–	-	7	2
CC398 (25)	t571 (13)	susceptible (2)	–	–	2	–
		ERY, CLI[a] (11)	*erm*(T) (10)	*eta* (1)	10	–
			erm(A), *erm*(T), *msr*(A) (1)	–	1	–
	t1451 (5)	ERY, CLI[a] (5)	*erm(T)* (3)	*eta* (1)	3	–
			erm(A), *erm*(T), *msr*(A) (1)	-	1	–
			erm(A), *erm*(T**)**, *lnu*(A) (1)	-	1	–
	t4030 (2)	ERY-I[b] (1)	*erm*(T**)** (1)	–	1	–
		ERY, CLI[a] (1)	*erm*(A), *erm*(T**)** (1)	–	1	–
	t034 (1)	ERY, CLI[a], TET (1)	*erm*(B), *msr*(A), *tet*(K) (1)	*eta* (1)	–	1
	t7880 (1)	ERY, CLI[a] (1)	*erm*(A), *erm*(T**)** (1)	–	1	–
	t011 (1), t1255 (1), t7160 (1)	susceptible (3)	-	–	2	1
CC45 (24)	t230 (7)	susceptible (6)	–	–	5	1
		TOB (1)	*ant(4)-Ia* (1)	–	–	1
	t015 (2)	susceptible (2)	–	*tst* (1)	1	1
	t026 (2)	susceptible (2)	–	*tst* (1)	2	–
	t330 (2)	ERY, CLI[a] (2)	*erm*(A), *msr*(A) (1)	–	2	–
			erm(A), *lnu*(A), *vga*(A) (1)	–		
	t502 (1)	susceptible (1)	–	*lukF/lukS-PV* (1)	1	–
	t065 (2), t287 (1), t362 (1), t445 (1), t808 (1), t1424 (1), t1601 (1), t3537 (1), t10988 (1)	susceptible (10)	–	-	5	5
CC8 (13)	t008 (4)	susceptible (4)	–	*lukF/lukS-PV* (1)	4	–
	t2558 (2)	TOB, CIP, FUS (1)	*ant(4)-Ia* (1)	–	–	1
		susceptible (1)	–	–	–	1
	t211 (1)	susceptible (1)	–	*etb* (1)	–	1
	t620 (2), t024 (1), t148 (1), t197 (1), t5160 (1)	susceptible (6)	–		4	2
CC97 (8)	t359 (2)	susceptible (2)	–	*lukF/lukS-PV* (1)	2	–
	t267 (2), t224 (1), t1190 (1), t1236 (1), t2802 (1)	susceptible (6)	–	–	5	1
CC1 (4)	t189 (3), t177 (1)	susceptible (4)	–	–	2	2
CC12 (3)	t156 (1), t183 (1), t3938 (1)	susceptible (3)	–	-	2	1
CC15 (3)	t346 (2), t7044 (1)	susceptible (3)	–	-	1	2
CC6 (2)	t701 (1)	susceptible (1)	–	–	1	–
	t7966 (1)	CIP (1)	–	–	–	1
CC30 (2)	t012 (1), t7561 (1)	susceptible (2)	–	–	2	–
CC59 (2)	t216 (1), t2597 (1)	susceptible (2)	–	–	–	2
CC121 (2)	t272 (1), t645 (1)	susceptible (2)	–	*eta* (1)	2	–

Continued

Table 1. *Continued*

CC	*spa* types (no. of isolates)	Resistance phenotype (no. of isolates)	Resistance genotype (no. of isolates)	Virulence genes (no. of isolates)	IEC[e]	
					presence	absence
Unknown (6)	t4558 (2), t14122 (2), t18816[c] (2)	susceptible (6)	–	–	6	–
Other (22)	others[d] (22)	susceptible (19)	–	*tst* (3)	16	3
		ERY, CLI[a] (1)	*erm*(A) (1)	-	–	1
		MUP (1)	*mup*(A) (1)	-	1	–
		TOB (1)	*ant(4)-Ia* (1)	-	–	1

ERY, erythromycin; CLI, clindamycin; TET, tetracycline; TOB, tobramycin; CIP, ciprofloxacin; FUS, fusidic acid; MUP, mupirocin.
[a]Inducible resistance.
[b]I, intermediate.
[c]New *spa* type.
[d]Twenty-two *spa* types; each *spa* type was only associated with one isolate (presumptive CC is indicated when possible): t005-CC22, t056-CC101, t240-CC10, t209-CC9, t355-CC152, t992, t1083, t1976, t2168, t2218, t2264, t2778, t3698, t4704, t5078-CC75, t5614, t7139, t10581, t15580, t18814[c], t18815[c] and t18944[c].
[e]The number of isolates that were positive or negative for IEC is shown.

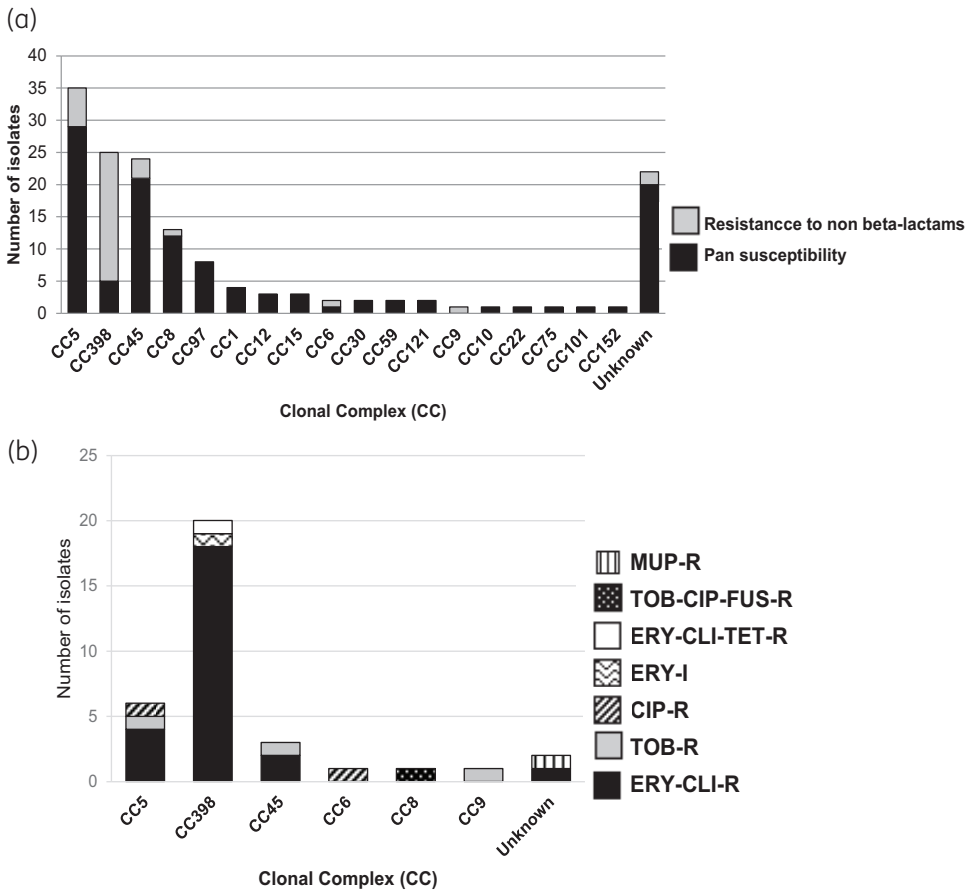

Figure 1. Relationship between CCs and antimicrobial resistance phenotype detected among the 151 MSSA-PEN^S-*blaZ*^{negative} isolates. (a) Pan-susceptible isolates or isolates resistant to non-β-lactams detected among the different CCs identified in all MSSA-PEN^S-*blaZ*^{negative} isolates. (b) CCs detected among isolates showing resistance to non-β-lactams and the antimicrobial resistance phenotype identified in those isolates. MUP-R, mupirocin resistance; TOB-CIP-FUS-R, tobramycin-ciprofloxacin-fusidic acid resistance; ERY-CLI-TET-R, erythromycin-clindamycin-tetracycline resistance; ERY-I, erythromycin intermediate resistance; CIP-R, ciprofloxacin resistance; TOB-R, tobramycin resistance; ERY-CLI-R, erythromycin-clindamycin resistance.

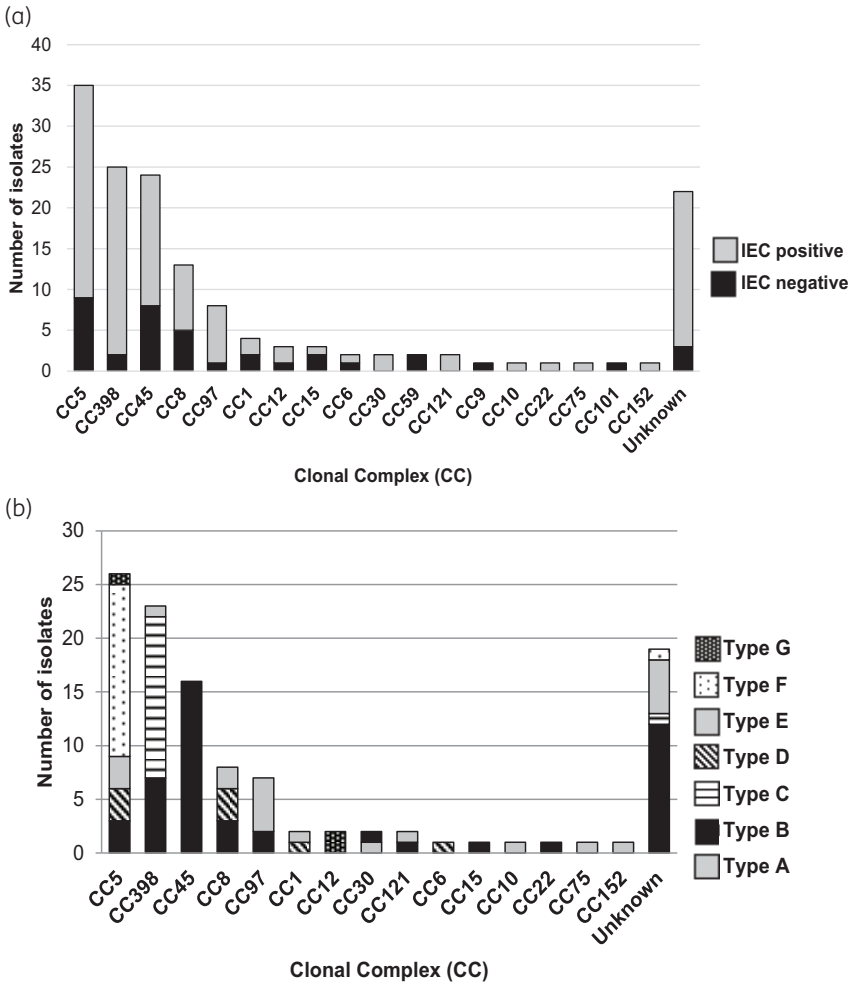

Figure 2. Relationship between CCs and the presence of an IEC detected among the 151 MSSA-PEN^{S}-*blaZ*^{negative} isolates. (a) Presence or absence of an IEC among the different CCs identified in all MSSA-PEN^{S}-*blaZ*^{negative} isolates. (b) IEC types detected among IEC-positive isolates according to their CCs.

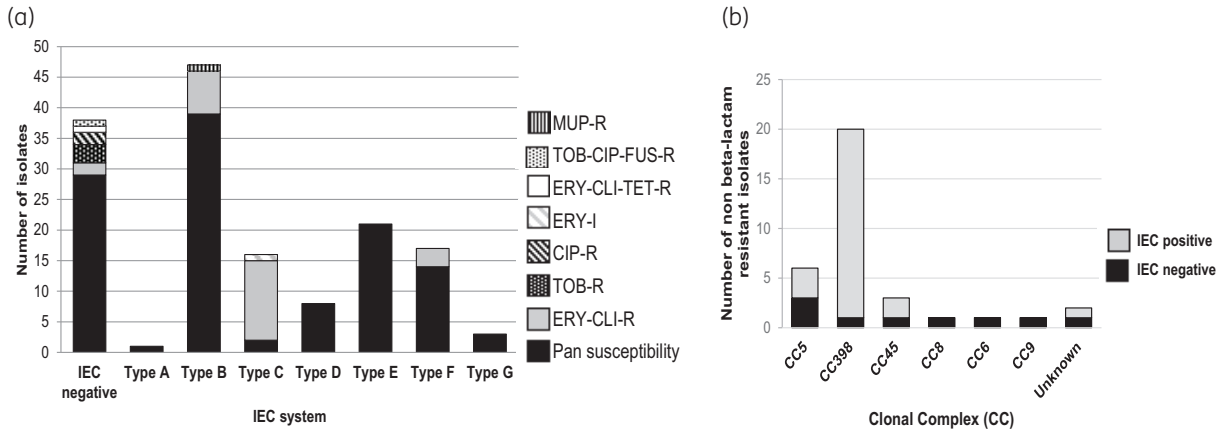

Figure 3. Relationship between IEC, antimicrobial resistance phenotype and CCs detected among the 151 MSSA-PEN^{S}-*blaZ*^{negative} isolates. (a) IEC types detected among the different antimicrobial resistance phenotypes identified in all MSSA-PEN^{S}-*blaZ*^{negative} isolates. (b) Presence or absence of an IEC among isolates showing resistance to non-β-lactams according to their CCs. MUP-R, mupirocin resistance; TOB-CIP-FUS-R, tobramycin-ciprofloxacin-fusidic acid resistance; ERY-CLI-TET-R, erythromycin-clindamycin-tetracycline resistance; ERY-I, erythromycin intermediate resistance; CIP-R, ciprofloxacin resistance; TOB-R, tobramycin resistance; ERY-CLI-R, erythromycin-clindamycin resistance.

Table 2. Characteristics of the four MSSA-PEN^{S}-*blaZ*^{positive} isolates obtained from blood cultures from 16 hospitals in Spain

Isolate	CC-*spa* type	Resistance phenotype	Penicillin MIC (mg/L)		Disc diffusion, 1 U penicillin disc (diameter mm)[b]	Nitrocefin	*blaZ* type[c]	Amino acid changes detected in BlaZ[d]	IEC type
			microdilution[a]	Etest					
X1953	CC5-t002	susceptible	≤0.12	0.016	26	negative	C	I86V, G145E, E193K, K196N, L203F, S207N, S217P, C220Y	F
X973	CC5-t148	susceptible	0.12	0.094	30	negative	B	I86V, E112A, T119K K131N, V139I, Q141K, G145K, E193K, K196N, S207N, S217P, C220Y, V241I, G245N, V251I	B
X933	CC5-t258	susceptible	≤0.12	0.016	42	negative	A	I86V, E112A, G145E, S217P, C220Y	B
X1992[e]	CC398-t571	ERY, CLI[f]	≤0.12	0.047	40	negative	C	I86V, G145E, E193K, K196N, L203F, S207N, S217P, C220Y	B
X1005	unknown-t18637[g]	susceptible	≤0.03	0.032	37	negative	C	I86V, G145E, E193K, K196N, L203F, S207N, S217P, C220Y	B

ERY, erythromycin; CLI, clindamycin.
[a]Determined by automatic methods used in the different hospitals.
[b]The halo was fuzzy in all cases.
[c]The type of BlaZ was defined by amino acids located at positions 119 and 207.
[d]Reference sequence from isolate *S. aureus* ATCC 29213.
[e]This strain carried the *erm*(C), *erm*(T), *lnuA* and *vgaA* genes.
[f]Inducible resistance.
[g]New *spa* type.

β-lactamases.[17] Penicillin resistance in *mecA*-negative *S. aureus* isolates is due to the production of these enzymes encoded by the *blaZ* gene and hence the absence of the *blaZ* gene would avoid this problem. Moreover, theoretically, the use of penicillin could also be useful not only in infections caused by MSSA but also in some infections caused by MRSA. It has been demonstrated that the *mecC* gene does not mediate resistance to penicillin[24] and that mutations in the *mecA* promoter region could confer susceptibility to the combination of this antimicrobial with β-lactamase inhibitors.[25] Nevertheless, in order to take advantage of the full potential of penicillin, it is necessary to improve the detection of penicillin-susceptible isolates at the laboratory level. In our study, most of the MSSA isolates identified as penicillin susceptible were *blaZ*^{negative} (96.8%). Percentages around 6%–7% of false penicillin-susceptible isolates have been described by others.[8,9,26] A penicillin MIC of ≤0.03 mg/L has been related to the absence of the gene *blaZ*.[8,13] However, in our study, two *blaZ*^{positive} isolates had an MIC value of 0.016 mg/L. Interestingly, different amino acid changes were identified in BlaZ among our MSSA-PEN^{S}-*blaZ*^{positive} isolates. Different studies have analysed the effect of *blaZ* types and polymorphisms at specific amino acid positions of BlaZ on the CzIE.[17] In addition, 34 changes in *blaRI* were detected. However, the role of these changes in penicillin susceptibility should be explored in the future. Remarkably, revertants were detected in two of our MSSA-PEN^{S}-*blaZ*^{positive} isolates. The potential use of penicillin to treat infections caused by these isolates could be affected by the presence of the *blaZ* gene.

Different clonal lineages were identified among our MSSA-PEN^{S}-*blaZ*^{negative} isolates, with the most prevalent being CC5 (23.2%), CC398 (16.6%) and CC45 (15.9%). CC5 is one of the CCs most commonly detected among human clinical MRSA and MSSA isolates in Europe.[27] Regarding MSSA, CC5 and/or CC45 have also been identified as dominant among penicillin-susceptible isolates by others.[13,24,28] CC398 was the second most frequently found clonal lineage among our isolates. CC398 MSSA-PEN^{S}-*blaZ*^{negative} isolates were also found in a previous study in Spain.[13] The CC398 lineage has been strongly linked to livestock-associated (LA) MRSA isolates.[14] However, half of our CC398 isolates (52%, 13/25) belonged to *spa* type t571, which has been identified as a human-adapted CC398 MSSA clade.[15,29] Moreover, only two CC398 isolates (the only ones with *spa* types t034 and t011) did not contain the *scn* gene, suggesting an animal origin.[29] It is important to note that CC398 MSSA has recently been identified as a human-adapted emerging clade in invasive infections in Spanish hospitals and also in French hospitals, both countries being geographically close.[15,30] According to that and considering our results, the appearance and spread of CC398 MSSA might be responsible, at least in part, for the increased rate of penicillin susceptibility detected in BSIs in Spain.

The gene *scn* was absent in 38 of our MSSA-PEN^{S}-*blaZ*^{negative} isolates (25.2%). Lack of the IEC system has been related to an animal origin.[14] In our study, IEC-negative isolates belonged to very diverse CCs (CC1, CC5, CC6, CC8, CC9, CC12, CC15, CC45, CC56, CC97, CC101 and CC398). Some of them, such as CC1, CC5, CC8,

CC9 and CC97, have been associated with LA-MRSA, having been detected in several animal species, such as pigs, poultry, sheep and cattle.[31,32] Nevertheless, there is little information about these clonal lineages among MSSA isolates.

Pan-susceptibility was identified in most of the isolates (77.5%). Resistance to non-β-lactam antimicrobials was also infrequent among MSSA-PEN^{S}-*blaZ*^{negative} isolates in other studies.[5,26] Among the antimicrobial resistance phenotypes detected, erythromycin resistance (17.9%) was the most common, with most of the erythromycin-resistant isolates belonging to CC398 (13.2%). In many of these isolates, this resistance was associated with the *erm*(T) gene, as already observed.[15] Remarkably, the only two MDR isolates were IEC negative, with one of them belonging to CC398. Some resistance phenotypes were only detected in IEC-negative isolates. Thus, all aminoglycoside-, quinolone- and/or tetracycline-resistant isolates lacked IEC genes, suggesting an association between these resistance phenotypes and a possible animal origin in MSSA-PEN^{S} isolates. However, macrolide, lincosamide and mupirocin resistances were mainly identified in IEC-positive isolates belonging to types B, C and/or F. Interestingly, a high prevalence of penicillin susceptibility has been previously detected among *S. aureus* isolates obtained from animal samples and these animal MSSA-PEN^{S} isolates showed resistance mainly to macrolides, tetracycline and/or aminoglycosides;[16,33] the low penicillin resistance in isolates from animals detected in those studies could be explained by the limited use of β-lactams on farm animals.[33]

In conclusion, increased penicillin susceptibility among invasive MSSA isolates in our country was confirmed. Although variable, in some hospitals more than a quarter of the MSSA isolates were susceptible to this antimicrobial. The reason for the re-emergence of penicillin-susceptible isolates is currently unclear. It may be due to the prescription of broad-spectrum antimicrobials by clinicians over recent decades because of traditionally high rates of penicillin resistance in *S. aureus* or the lack of confidence in current procedures for detecting penicillin susceptibility in the clinical microbiology laboratory since it was a 'forgotten' antimicrobial. Another possible explanation is the emergence of new successful penicillin-susceptible *S. aureus* clones. Resistance to non-β-lactam antimicrobials was mainly associated with CC398 and some IEC-negative isolates, which raises questions about their possible animal origin. Considering the progressive increase in MSSA in bacteraemia, penicillin might once again be considered as a possible antimicrobial for the treatment of *S. aureus* BSIs. Moreover, the study of the absence of *blaZ* in these penicillin-susceptible isolates might also allow the effective use of cefazolin for treating these infections.

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Transparency declarations

None to declare.

Author contributions

C.T. and C.A. conceived and designed the study. C.A., J.M.A., C.S., E.C., L.L.-C., P.P., A.B.-B., P.B., M.S. and A.A.-Q. (and the members of the Spanish Study Group of Clinical *S. aureus* CC398) designed and participated in the strain recovery, identification and susceptibility testing. O.M.M. and L.R.-R. performed the molecular characterization of isolates and susceptibility testing. C.T., C.A., C.L., O.M.M. and M.Z. interpreted the results and wrote the first draft of the manuscript. All authors reviewed and approved the manuscript.

Supplementary data

Table S1 and Figure S1 are available as Supplementary data at *JAC* Online

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