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Brief report

Penicillin-susceptible *Staphylococcus aureus* bacteremia: Epidemiological and clinical relevance. Possible therapeutic implications☆

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ABSTRACT

Introduction: The increase in penicillin susceptibility among *Staphylococcus aureus* (SA-Pen^S) might have therapeutic relevance. We aimed to study the current situation in our environment.
Material and methods: Over a 2.5 years period, all SA isolates from bacteraemia were analysed. For all isolates, antimicrobial susceptibility profile, beta-lactam resistance genes (*blaZ, mecA*) and Panton-Valentine leucocidine encoding-genes were studied. For SA-Pen^S-*blaZ*^{negative} isolates, *spa*-type, MLST and the presence of other resistance genes were studied.
Results: Among 84 patients with SA bacteraemia (35.7% MRSA and 64.3% MSSA), 77 were analysed; 22.2% of MSSA isolates were Pen^S and *blaZ*^{negative} (Pen-MIC ≤0.03 μg/mL) corresponding to 14.3% of the total SA. In MSSA-Pen^S-*blaZ*^{negative} isolates, eight *spa*-types corresponding to seven clonal complexes were detected.
Conclusion: A high prevalence of MRSA/SA and MSSA-Pen^S-*blaZ*^{negative}/MSSA was detected in blood cultures. Pen-MIC ≤0,3 μg/mL corresponded to MSSA-Pen^S-*blaZ*^{negative}. This situation raises therapeutic options which should be further evaluated in larger studies and clinical trials.

Bacteriemia por *Staphylococcus aureus* sensible a penicilina. Importancia epidemiológica, clínica y posibles implicaciones terapéuticas

Palabras clave:
Penicilina
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RESUMEN

Introducción: El aumento de la sensibilidad a penicilina en *Staphylococcus aureus* (SA-Pen^S) podría tener relevancia terapéutica. Pretendemos conocer esta situación en nuestro medio.
Material y métodos: Se analizaron bacteriemias por SA durante 2,5 años (2015–2017). Estudiamos la sensibilidad a antimicrobianos, genes de resistencia a beta-lactámicos (*blaZ, mecA*) y presencia de leucocidina de Panton-Valentine. En aislados SA-Pen^S-*blaZ*^{negativo} se determinó el tipo de *spa*, MLST y genes de resistencia a antimicrobianos no-beta-lactámicos.
Resultados: Hubo 84 pacientes con bacteriemia por SA (35,7% SARM y 64,3% SASM), se analizaron 77. El 22% de los SASM estudiados (n = 11) fueron Pen^S- *blaZ*^{negativo} (CMI-Pen ≤0,3 μg/mL), correspondiendo a 14,3% del total de SA. En SASM-Pen^S-*blaZ*^{negativo} se detectaron ocho tipos de *spa* asociados a siete complejos clonales.

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Conclusión: Detectamos alta prevalencia de SARM/SA y de SASM-Pen^S-*blaZ*^{negativo}/SASM en hemocultivos. Una CMI-Pen ≤0,3 μg/mL se correspondió con SASM-Pen^S-*blaZ*^{negativo}. Esta situación plantea opciones terapéuticas que deberán reevaluarse con estudios más amplios y ensayos clínicos.

Introduction

Staphylococcus aureus (SA) bacteraemia is an entity with special relevance due to its frequency, severity and high mortality. In the treatment of Methicillin-susceptible SA (MSSA) bacteraemia, the timeliness of suitable treatment is very important. In targeted therapy there is controversy about the use of different beta-lactams, with cloxacillin and cefazolin as first-line, and poorer results with second and third generation cephalosporins and beta-lactams with beta-lactamase inhibitors.[1] Regarding penicillin, due to its high resistance, it is not generally used for these treatments, especially in bacteraemia. However, in addition to the vast experience with this antibiotic over decades, recent works indicate that it still has points in its favour for even standing as a treatment of choice for SA infections susceptible to this antibiotic (SA-Pen^S).[2–4] These points include: 1) MIC values 10–50 times lower and a lower percentage of protein binding than cloxacillin; 2) the time above the MIC is longer than other antimicrobials, including cefazolin[5]; 3) narrower spectrum than other options, including first-generation cephalosporins (thus related to fewer infections by *Clostridium difficile*),[6] and 4) less selection of *mecA* and *mecC*-mediated methicillin resistance.[7]

In various countries around the world there is a growing trend in penicillin-sensitive SA, especially in invasive infections like bacteraemia. Data from the USA,[6,8–10] Canada,[4] Europe (Sweden,[5,11] Finland[12] and Denmark[2]) or from Australia and New Zealand[3] exemplify this phenomenon, including penicillin in a kind of ẗecond chance.[4]

Penicillin resistance in *mecA*-negative SA strains is due to the production of beta-lactamases encoded by the *blaZ* gene. There are several phenotypic methods for its detection, such as the nitrocefin test or the reading of the halo edge of penicillin on disk-diffusion, but compared with PCR detection of the *blaZ* gene they have less sensitivity.[7,9,11]

The objective of this work was to understand the situation of penicillin sensitivity in cases of bacteriaemia caused by SA and its characteristics in our environment.

Material and methods

During the study period (July 2015-December 2017) SA strains isolated from bacteraemia (both MSSA and methicillin-resistant *Staphylococcus aureus* [MRSA]) were analysed, in adult patients at the Hospital Royo Villanova [Royo Villanova Hospital], Zaragoza. The blood cultures (Bactec, Becton, Dickinson®) and the sensitivity study (MicroScan, Beckman® Combo 31 panel) were performed following the usual *European Committe for Antimicrobial Susceptibility Testing* protocols and cut-off points.

In all the SA strains (one per patient) the presence of the *blaZ* and *mecA*genes was analysed, as well as the Panton-Valentine leukocidin (PVL) gene.[13]

The characterisation of the *blaZ*-negative (*blaZ*^{negative}) isolates was obtained through: 1) *spa* typing by PCR and sequencing[13]; 2) determination of the sequence type (ST) and clonal complex (CC) by the multilocus-sequence typing (MLST) technique from a representative isolate of each of the different *spa*-types detected, assuming the ST for all the isolates of the same*spa,*[13] and 3) PCR study of non-beta-lactam antibiotic resistance genes: macrolides (*ermA*/*ermB*/*ermC*/*msrA*/*msrB* y *ermT*), tobramycin (*ant(4)'-Ia*) or mupirocin *(mupA).*[13]

Results

Over the 30 months of study there were 84 patients with SA bacteraemia (30 with MRSA [35.7%] and 54 with MSSA [64.3%]). Of the MSSA isolates, 12 were susceptible to penicillin (MSSA-Pen^S), representing 22.2% of MSSA and 14.3% of SA.

Of the total of 84 SA, 77 isolates were recovered for characterisation (27 MRSA and 50 MSSA). The 27 MRSA isolates were resistant to penicillin (MIC: >0.12mcg/m), carried the *mecA* and *blaZ* genes, and were negative for the PVL gene.

The 50 MSSA isolates lacked *mecA*, and in relation to penicillin: a) 39 of them showed phenotypic resistance to penicillin (MIC: >0.25 μg/mL) and contained the *blaZ* gene (MSSA-Pen^R-*blaZ*^{positive}), and b) 11 were susceptible to penicillin and lacked the *blaZ* gene (MSSA-Pen^S-*blaZ*^{negative}), all of them showing an MIC-Pen≤0.3 μg/mL (Table 1).

The 11 MSSA-Pen^S-*blaZ*^{negative} isolates were assigned to 8 different *spa* -types (t002 the most frequent), associated with 8 STs and 7 CCs (CC5, CC8, CC22, CC45, CC59, CC97 y CC398). Six of the 11 MSSA-Pen^S-*blaZ*^{negative} isolates were susceptible to all the antibiotics tested and the remaining 5 presented resistance to macrolides, lincosamides, tobramycin, mupirocin and/or fluoroquinolones (genes *ermA*, *ermT*, *msrA*, *msrB*, *ant(4)'-Ia* or *mupA*being detected). Lastly, one of the MSSA-Pen^S-*blaZ*^{negative} strains was a carrier of the PVL gene (t121-ST8/CC8), and only resistant to fluoroquinolones.

Discussion

In this study of SA bacteraemia, a MRSA prevalence of 35.7% was detected with 22.2% susceptibility to penicillin among MSSA isolates (14.3% of total SA). The percentage of MRSA is higher than that of other European countries and that of most Spanish hospitals (https://ecdc.europa.eu/en/about-us/partnerships-and-networks/disease-and-laboratory-networks/ears-net).

Regarding the MSSA-Pen^{S}isolates, published figures are between 20 and 33% in northern European countries,[5,11] USA[9,14] or Canada.[4,10] In Spain we have not found any recent data, but in a multicentre study[15] with 20-year evolution data (1986–2006) the results of 6 prevalence studies in Spanish hospitals were analysed and it was observed that there was an increase in global susceptibility to penicillin in SA isolates from different clinical samples, with the lowest figures in 1991 (2.9%) and a sustained increase up to 2006 (11.0%). The data of our series of 14.3% global and 22.2% in MSSA would be in line with an increase in global susceptibility to penicillin in SA.

Of all the Pen^S strains characterised, (n = 11), all presented MIC-Pen≤0.3 μg/mL values and lacked the *blaZ* gene. This observation contributes to simplifying the microbiological diagnosis of penicillin susceptibility determination, since PCR detection of the *blaZ* gene is not available in most laboratories, the interpretation of susceptibility by disk-diffusion is not very objective and other tests such as nitrocefin are not recommended. Our data coincide with other authors that demonstrate that in strains with high susceptibility to penicillin (<0.03 μg/mL) the presence of beta-lactamases mediated by *blaZ*[1,9] can be reliably ruled out. Clonal complexes CC5,

Table 1
Phenotypic and genetic characteristics of 11 strains of *Staphylococcus aureus* Pen^{-S}-*blaZ*^{negative} isolated from blood cultures.

Isolate code	ST/CC	*Spa* -type	Antibiotic resistance		Virulence genes	Clinical picture/focus	Origin of the infection
			Resistance phenotype	Resistance genes			
X797	ST5/CC5	t002	ERI-CLI[a]	*msrA/msrB, ermA*	—	RTI	HAI
X748	ST5/CC5	t002	ERI-CIP-LEVO-MUP	*msrA/msrB*	—	SSTI. Cellulitis	Community
X728	ST5/CC5	t002	Pansusceptible[b]	—	—	UTI	HAI
X770	ST1099/CC5	t2450	ERI-CIP-LEVO-MUP-TOB	*msrA/msrB, mupA, ant(4́)-Ia*	—	UTI	HAI
X737	ST8/CC8	t121	CIP-LEVO	—	*lukF/lukS-PV*	SSTI. Abscesses	Community
X749	ST22/CC22	t2816	Pansusceptible[b]	—	—	SSTI?	HAI
X727	ST45/CC45	Non-identifiable	Pansusceptible[b]	—	—	BAC	Nosocomial
X756	ST59/CC59	t216	Pansusceptible[b]	—	—	Fever without a focus. SSTI-PUI?	HAI
X766	ST97/CC97	t359	Pansusceptible[b]	—	—	Fever without a focus	HAI
X787	ST97/CC97	t1236	Pansusceptible[b]	—	—	RTI? SSTI-PUI?	HAI
X746	ST398/CC398	t571	ERI-CLI[a]	*msrA/msrB, ermT*	—	BAC	Nosocomial

?: probable, unproven infection; BAC: catheter-related bacteriaemia; CIP: ciprofloxacin; CLI: clindamycin; ERI: erythromycin; SSTI: skin and soft tissue infections; HAI: health care-associated infections; RTI: respiratory tract infection; UTI: urinary tract infection; LEVO: levofloxacin; MUP: mupirocin; TOB: tobramycin; PUI: pressure ulcer infection.

[a] Inducible resistance phenotype.

[b] Susceptible to the following antimicrobials: cefoxitin, oxacillin, penicillin, phosphomycin, rifampin, mupirocin, fusidic acid, gentamicin, tobramycin, amikacin, ciprofloxacin, levofloxacin, cotrimoxazole, vancomycin, teicoplanin, daptomycin, linezolid, and tetracycline.

CC8, CC45 y CC22 associated with our MSSA-Pen^{S}-*blaZ*^{negative} were also detected in a similar study in Sweden, with CC5-t002[11] also predominant.

An explanation for the increased susceptibility to penicillin in SA could be attributed to a lower selective pressure due to its lower use. In fact, there is a trend in this regard in the first years of the 21 st century compared to previous years, which is especially marked in the USA and Canada.[6,10] The only country reporting a downward trend is Sweden,[5] a country with an antibiotic policy that has prioritised narrow-spectrum antibiotics for years, which went from 57% susceptibility in 2008–09 to 29% in 2014–15. Perhaps it could be interpreted as an increasing phenomenon in countries where penicillin is not consumed and decreasing in those where it is used, with both situations coinciding with figures of 20-30% at the present time. Another possible explanation is that new, more epidemiologically successful SA clones are coming in to replace the previous ones, and among these some lack *blaZ*. This aspect should be monitored in the future.

One of the limitations of our study is that of being single-centre. It would be interesting to see if this prevalence of penicillin susceptibility (with absence of *blaZ*) in SA exists in other hospitals in our country.

There are no randomised studies comparing benzylpenicillin with other antistaphylococcal penicillins (cloxacillin) or with cefazolin, and its use has been so low since the end of the last century (due to increased resistance) that sufficient data has not been collected in retrospective studies. In some studies that analyse the use of penicillin, a higher mortality has not been observed compared with comparators such as dicloxacillin.[2,6] In one study,[6] the use of penicillin went from 0 to 50% of susceptible cases, with good results. Another recent article[3] shows a slightly higher mortality of flucloxacillin compared to benzylpenicillin, although with methodological limitations.

Pencillin susceptibility in MSSA expands targeted treatment options. The limited data on its usefulness is favourable, but randomised studies are needed to determine the most appropriate treatment. The narrowest of spectrums is one of the aspects that must take precedence in the treatment of infections, without ruling out other aspects such as administration dosage difficulties.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

References

1. Paul M, Zemer-Wassercug N, Talker O, Lishtzinsky Y, Lev B, Samra Z, et al. Are all beta-lactams similarly effective in the treatment of methicillin-sensitive *Staphylococcus aureus* bacteraemia? Clin Microbiol Infect. 2011;17:1581–6, http://dx.doi.org/10.1111/j.1469-0691.2010.03425.x.
2. Nissen JL, Skov R, Knudsen JD, Ostergaard C, Schønheyder HC, Frimodt-Møller N, et al. Effectiveness of penicillin, dicloxacillin and cefuroxime for penicillin-susceptible *Staphylococcus aureus* bacteraemia: a retrospective, propensity-score-adjusted case-control and cohort analysis. J Antimicrob Chemother. 2013;68:1894–900, http://dx.doi.org/10.1093/jac/dkt108.
3. Henderson A, Harris P, Hartel G, Paterson D, Turnidge J, Davis JS, et al. Benzylpenicillin versus flucloxacillin for penicillin susceptible *Staphylococcus aureus* bloodstream infections from a large retrospective cohort study. Int J of Antimicrob Agents. 2019, http://dx.doi.org/10.1016/j.ijantimicag.2019.05.020.
4. Cheng MP, René P, Cheng AP, Lee TC. Back to the Future: Penicillin-Susceptible *Staphylococcus aureus*. Am J Med. 2016;129:1331–3, http://dx.doi.org/10.1016/j.amjmed.2016.01.048.
5. Hagstrand Aldman M, Skovby A, I Påhlman L. Penicillin-susceptible *Staphylococcus aureus*: susceptibility testing, resistance rates and outcome of infection. Infect Dis (Lond). 2017;49:454–60, http://dx.doi.org/10.1080/23744235.2017.1280617.
6. Chabot MR, Stefan MS, Friderici J, Schimmel J, Larioza J. Reappearance and treatment of penicillin-susceptible *Staphylococcus aureus* in a tertiary medical centre. J Antimicrob Chemother. 2015;70:3353–6, http://dx.doi.org/10.1093/jac/dkv270.
7. Butler-Laporte G, Lee TC, Cheng MP. Increasing Rates of Penicillin Sensitivity in *Staphylococcus aureus*. Antimicrob Agents Chemother. 2018;62:e00680–18, http://dx.doi.org/10.1128/AAC.00680-18.
8. Koeck M, Como-Sabetti K, Boxrud D, Dobbins G, Glennen A, et al. Burdens of invasive methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* disease, Minnesota, USA. Emerg Infect Dis. 2019;25:171–4, http://dx.doi.org/10.3201/eid2501.181146.
9. Richter SS, Doern GV, Heilmann KP, Miner S, Tendolkar S, Riahi F, et al. Detection and prevalence of penicillin-susceptible *Staphylococcus aureus* in the United States in 2013. J Clin Microbiol. 2016;5:812–4, http://dx.doi.org/10.1128/JCM.03109-15.
10. Kanjilal S, Sater MRA, Thayer M, Lagoudas GK, Kim S, Blainey PC, et al. Trends in antibiotic susceptibility in *Staphylococcus aureus* in Boston, Massachusetts, from 2000 to 2014. J Clin Microbiol. 2017;56:e01160-17, http://dx.doi.org/10.1128/JCM.01160-17.
11. Resman F, Thegerström J, Månsson F, Ahl J, Tham J, Riesbeck K. The prevalence, population structure and screening test specificity of penicillin-susceptible *Staphylococcus aureus* bacteremia isolates in Malmö, Sweden. J Infect. 2016;73:129–35, http://dx.doi.org/10.1016/j.jinf.2016.05.011.
12. Jokinen E, Laine J, Huttunen R, Rahikka P, Huhtala H, Vuento R, et al. Comparison of outcome and clinical characteristics of bacteremia caused by methicillin-resistant, penicillin-resistant and penicillin-susceptible

Staphylococcus aureus strains. Infect Dis (Lond). 2017;49:493–500, http://dx.doi.org/10.1080/23744235.2017.1292046.
13. 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, http://dx.doi.org/10.1016/j.ijmm.2014.09.004.
14. Livorsi DJ, Crispell E, Satola SW, Burd EM, Jerris R, Wang YF, et al. Prevalence of *blaZ* gene types and the inoculum effect with cefazolin among bloodstream isolates of methicillin-susceptible *Staphylococcus aureus*. Antimicrob Agents Chemother. 2012;56:4474–7, http://dx.doi.org/10.1128/AAC.00052-12.
15. Cuevas O, Cercenado E, Goyanes MJ, Vindel A, Trincado P, Boquete T, et al. *Staphylococcus* spp. en España: situación actual y evolución de la resistencia a antimicrobianos. Enferm Infecc Microbiol Clin. 2008;26:269–77.