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Prevalence of pSCFS7-like vectors among cfr-positive staphylococcal population in Spain

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1 **Prevalence of pSCFS7-like vectors among *cfr* positive staphylococcal**
2 **population in Spain**

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31 Sir,

32 As a consequence of the dissemination of methicillin-resistant *Staphylococcus aureus*
33 (MRSA) strains and the accumulation of several resistant traits, only a few antibiotics such as
34 vancomycin or linezolid are considered as the last resort antibiotics for the treatment of these
35 strains. The finding of a transferable chloramphenicol/florphenicol resistance (*cfr*) gene,
36 related to linezolid resistance, has compromised the usage of this antimicrobial in the clinical
37 practice. Although the prevalence of MRSA strains showing resistance to linezolid is still low,
38 hospital outbreaks due to *cfr*-positive MRSA strains have been documented in Spain [1].

39 Among the different vectors related to *cfr* gene, pSCFS7-like plasmids have been detected in
40 various European countries, such as Ireland, Germany and Spain [2-4]. It has been
41 experimentally demonstrated that these ca. 40 kbp plasmids can be transferred by conjugation
42 from coagulase-negative staphylococcal strains (CoNS) or MRSA to MRSA strains, as well as
43 between MRSA isolates by the phage-associated transduction [4]. However, given the low
44 frequency of detection of these strains, no systematic study has been available to allow an in-
45 depth understanding of the dissemination of pSCFS7-like vectors in Spain. Thus, both the
46 epidemiology of pSCFS7-like plasmids in CoNS populations, and the correlation between these
47 vectors and those present in MRSA population in Spain remain to be fully elucidated. The main
48 objective of this work is to analyze the *cfr*-associated vectors in a collection of clinical
49 staphylococcal strains obtained from different and geographically unrelated Spanish hospitals,
50 in order to clarify the current scenario of *cfr* dissemination in this country.

51 In the present study, 39 staphylococcal *cfr*-positive strains have been analyzed. The bacterial
52 collection includes four species: *Staphylococcus epidermidis* (n=22), *Staphylococcus hominis*
53 (n=7), *Staphylococcus lugdunensis* (n=1) and MRSA (n=9). These strains have been obtained
54 among the staphylococci routinely studied and conserved in the Spanish National Centre of
55 Microbiology, and have been collected from 22 different and geographically unrelated Spanish
56 hospitals between 2012 and 2016 (Table S1, Figure S1). All of these strains are *cfr*-positive on
57 the basis of the PCR detection, and show a linezolid MIC ≥ 4 $\mu\text{g}/\text{mL}$. Substitutions in the 23S
58 rRNA gene related to linezolid resistance have been identified as previously described and the
59 presence of a pSCFS7-like plasmid in these strains has been tested by using a previously
60 described PCR-based system [4].

61 As summarized in table 1, 85% of the studied strains (33 among the 39 clinical *cfr*-positive
62 isolates studied) exhibit the presence of pSCFS7-like plasmids on the basis of the PCR analysis.

63 This result shows the prevalence of pSCFS7-like plasmids in the *cfr*-positive staphylococcal
64 strains in Spain.

65 Our list includes, to the best of our knowledge, the first report of a *cfr*-positive *S. lugdunensis*,
66 obtained in the hospital 7 (Table S1). The analysis comprises a total number of 11
67 staphylococcal isolates from this hospital (two MRSA, eight *S. epidermidis* and one *S.*
68 *lugdunensis*, table S2). All the strains harbor pSCFS7-like vectors, showing that the theoretical
69 reservoir and its final receptor share the same type of conjugative *cfr*-plasmids. Our results
70 show the spread of pSCFS7-like plasmids within this hospital. It is conceivable that pSCFS7-like
71 plasmids can disseminate among the clinical CoNS pool, once they arrived to the clinical
72 settings, rendering themselves accessible for the other CoNS and MRSA strains. In this regard,
73 an outbreak of *cfr*-positive MRSA strains was reported in one Spanish hospital in 2010 [1]. Four
74 years later, more than 50% of the linezolid-resistant *S. epidermidis* isolates in this hospital
75 were *cfr* positive [5].

76 Although pSCFS7 plasmids are prevalent among the clinical staphylococcal population
77 analyzed, 6 strains (15 %) harbor *cfr* on non-pSCFS7 plasmids, showing that pSCFS7 is not the
78 only vector involved in linezolid resistance in Spain. These strains show negative results for the
79 detection of other previously described *cfr*-associated vectors, such as p12-00322, pSCFS6,
80 pSCFS3 or pERGB (data not shown). Thus, the presence of classical *cfr* vectors-related genes,
81 such as IS21 sequences, Tn558 transposon genes (*tnpA*, *tnpB* and *tnpC*) and *fexA* gene has
82 been analyzed in pSCFS7 negative strains by PCR (Table S2). We have found different
83 amplification profiles in the six strains studied (Table S3). Although this system does not allow
84 us to further characterize unknown vectors, the different amplification profiles suggest the
85 existence of diverse plasmids in these strains.

86 In summary, pSCFS7-like plasmids are prevalent among *S. aureus*, *S. epidermidis* and *S. hominis*
87 isolated in Spanish hospitals, and minor uncharacterized plasmids are also found. In addition,
88 we report the first *cfr*-positive *S. lugdunensis* harboring a pSCFS7-like plasmid. These results
89 support the crucial role of non-pathogenic bacteria in the evolution of pathogenic species.
90 Since these bacteria act as genetic reservoirs, this study emphasizes the importance to include
91 CoNS species in the surveillance programs, in the context of the WHO global action plan on
92 antimicrobial resistance (AMR), aiming to improve the knowledge on detection, effective
93 infection control and surveillance.

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99 **Transparency declarations**

100 None to declare

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102 **References**

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