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Evaluation of the presence of the *bap* gene in *Staphylococcus aureus* isolates recovered from human and animals species

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28 **Abstract:**

29 The implication of biofilm in chronic bacterial infection in many species has triggered
30 an increasing interest in the characterization of genes involved in biofilm formation. The *bap*
31 gene is a newly identified gene that encodes the biofilm-associated protein, BAP, which is
32 involved in biofilm formation in *Staphylococcus aureus*. So far the *bap* gene has only been
33 found in a small proportion of *S. aureus* strains from bovine mastitis in Spain. In order to
34 study the presence of the *bap* gene in *S. aureus* isolates obtained from other species and
35 various locations, a collection of 262 isolates was tested by PCR, using published primers
36 and dot-blot. The results indicated that none isolates carried the *bap* gene suggesting that
37 the prevalence of this gene among *S. aureus* isolates should be very low.

38 **Keywords:** *Staphylococcus aureus*; *Bap* gene; Biofilm; Epidemiology

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53 **1. Introduction**

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55 In *Staphylococcus aureus* (*S. aureus*) the implication of biofilm in chronic infections in
56 all animal species have triggered an increasing interest in the characterization of genes

57 involved in this biofilm formation. For example, the biofilm formation is important for virulence
58 in mastitis (Baselga et al., 1993). A new gene (6,831 nucleotides) involved in biofilm
59 formation (*bap* coding for a biofilm-associated protein, Bap) was identified in a small
60 proportion of *S. aureus* from bovine mastitis (Cucarella et al., 2001). The *bap* protein is a
61 member of proteins playing a role in biofilm formation in many bacteria. They share common
62 structural features as they have a high molecular weight and contain a core domain of
63 tandem repeats. These proteins confer upon bacteria the capacity to form a biofilm and play
64 a relevant role in bacterial infectious process. Some of these proteins are contained
65 occasionally in mobile elements (Lasa and Penades, 2006). In *S. aureus*, the *bap* gene is
66 carried by a putative composite transposon inserted in SaPIbov2, a mobile staphylococcal
67 pathogenicity island. *Bap* orthologue genes have been found in other staphylococcal species
68 including *Staphylococcus epidermidis*, *Staphylococcus chromogenes*, *Staphylococcus*
69 *xylosus*, *Staphylococcus simulans* and *Staphylococcus hyicus*. However, sequence analyses
70 of the flanking regions revealed that these orthologue *bap* genes of these staphylococcal
71 species were not contained in the SaPIbov2 pathogenicity island (Tormo et al., 2005).

72 The aim of the present study was to investigate the presence of *bap* gene in various
73 *S. aureus* isolates recovered from human and different animal species. To do this a
74 published PCR method was used (Cucarella et al., 2001) and results were confirmed by dot
75 blot analysis.

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77 **2. Materials and methods**

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79 2.1. *S. aureus* isolates used in the study

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81 Two hundred and sixty two *S. aureus* isolates associated with different diseases were
82 recovered from various locations in France and different animal species (cows, sheep, goats,
83 pigs, rabbits, poultry, horses, human) (table 1). The cow's isolates were a gift from Dr J.L
84 Martel (AFSSA Lyon). Some sheep isolates were from ML De Buyser (AFSSA Maisons-
85 Alfort). The goat's isolates were partly from Dr P. Mercier (AFSSA Niort). The pig, poultry and

86 rabbits isolates were from M.H. Bâyon-Auboyer (Departemental Laboratory Côtes d'Armor).
87 The horse's isolates were a gift from Dr C. Collobert (AFSSA Dozulé). The human isolates
88 were mainly from Dr H. Carsenti-Dellamonica (Hospital of Archet, Nice, France). All the
89 remaining isolates were from AFSSA Sophia-Antipolis.

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92 2.2. Detection of the *bap* gene by PCR

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94 DNA extraction was performed using the DNeasy[®] Tissue Kit (Qiagen, Courtaboeuf,
95 France) according the manufacturer's instructions with slight modifications. Lysostaphin
96 (Sigma, St Quentin, France) were added (1mg/ml) for enzymatic lysis at 37°C for 2 h.

97 PCR were performed twice, using a primer pair (*sasp-6m* : 5'
98 CCCTATATCGAAGGTGTAGAATTGCAC 3' and *sasp-7c* : 5'
99 GCTGTTGAAGTTAATACTGTACCTGC 3') as described by Cucarella (Cucarella et al.,
100 2004) to detect the *bap* gene. Amplification was carried out on a Mastercycler[™] (Eppendorf,
101 Hamburg, Germany) with Platinum[®] Taq DNA Polymerase (Invitrogen, Cergy Pontoise,
102 France) under the following conditions: an initial 2 minutes denaturation step at 94°C;
103 followed by 40 cycles each of 30 seconds at 94°C, 30 seconds at 55°C, and 75 seconds at
104 72°C; and a final step at 72°C for 5 minutes. A 971-bp PCR fragment was expected.

105 The primer pair (*staur4* : 5' ACGGAGTTACAAAGGACGAC 3' and *staur6* : 5'
106 AGCTCAGCCTTAACGAGTAC 3') was used to target the 23S rDNA as described by Straub
107 (Straub et al., 1999) to confirm the quality of each DNA extract and the absence of PCR
108 inhibitor. The following conditions were used: an initial 5 minutes step at 94°C; followed by 30
109 cycles each consisting of 30 seconds at 94°C, 30 seconds at 58°C, and 75 seconds at 72°C;
110 and a final step at 72°C for 5 minutes. A 1250-bp PCR fragment was expected.

111 A *bap* positive control strain V329 (Genbank accession no. AY220730, kindly
112 provided by Dr J.R. Penadés, Spain) was used with each PCR run. Amplification products
113 were electrophoresed in a 1% agarose gel containing ethidium bromide and visualized by
114 transillumination under UV light.

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116 2.3. Dot blotting and hybridisation for the *bap* gene

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118 For the dot blotting, 141 isolates were randomly chosen among the two hundred and
119 sixty two isolates.

120 The dot blotting technique was described by Planchon et al. (Planchon et al., 2006).
121 Briefly, 50 ng of denatured DNA were spotted onto N + nylon membrane and treated
122 according to the manufacturer's instructions (Amersham Biosciences, Buckinghamshire,
123 England). The PCR product amplified with the primers *sasp-6m* and *sasp-7c* (Cucarella et
124 al., 2004), specific for the *bap* gene (971 bp long) from the V329 *S. aureus* strain, was used
125 as the probe. This PCR product was purified with a QIAquick[®] PCR purification kit (Qiagen,
126 Courtaboeuf, France), labelled with the DIG-High Prime[®] system (Roche, Neuilly sur Seine,
127 France). The hybridisations were done in DIG Easy Hyb[®] solution and the hybridised probe
128 was detected by the Dig colour detection[®] kit (Roche, Neuilly sur Seine, France) following the
129 manufacturer's instructions. *S. aureus* V329 (Genbank accession no. AY220730) was used
130 as positive control and the strain Mu50 (Genbank accession no. BA000017) as negative
131 control.

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133 3. Results

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135 DNA extracted from 262 *S. aureus* isolates was tested for the presence the *bap* gene
136 by PCR, using the primer pair *sasp-6m* and *sasp-7c*, as indicated in the materials and
137 methods section. Although the positive control strain (V329) showed a band at 971 bp, as
138 expected, none of the tested isolates showed positive results. All isolates were also tested
139 for the presence of the *S. aureus* 23S DNA by using PCR to check for DNA quality, presence
140 of inhibitors of the PCR reactions and specificity. All isolates were found positive, thus
141 eliminating false negative results. An example of some PCR results is shown in figure 1.

142 Since it cannot be excluded that mutations or deletions could have occurred in the
143 primer pair region of the *bap* gene, 141 isolates were randomly selected and tested by dot

144 blotting. As illustrated in figure 2, all isolates were also found negative by using this
145 technique, except the *bap* positive control strain V329. Therefore it is likely that the *bap* gene
146 is lacking in all isolates of *S. aureus* tested in this study.

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149 **4. Discussion**

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151 The gene for the biofilm associated protein (*bap* gene) was not detected in the twenty
152 hundred and sixty two *S. aureus* isolates of this study. These results are in agreement with
153 previous surveys on *S. aureus* of human, bovine, rabbit and pig origins (Arciola et al., 2001;
154 Vasudevan et al., 2003; Vancraeynest et al., 2004; Nitzsche et al., 2007) where the *bap* gene
155 was not found in the *S. aureus* isolates recovered in these animal species of these studies.
156 Our study is the first one with a wide range of *S. aureus* recovered from different animal
157 species to show that the *bap* gene had not spread yet among *S. aureus*.

158 So far, the *bap* gene has only been found in *S. aureus* obtained from bovine
159 subclinical mastitis in Spain (Cucarella et al., 2001). This gene is also present in other
160 *Staphylococcus* species, including *S. epidermidis*, *S. chromogenes*, *S. xylosus*, *S. simulans*
161 and *S. hyicus* (Tormo et al., 2005; Planchon et al., 2006). But, the *bap* gene is not widely
162 distributed in *S. aureus* isolates despite its presence in the pathogenicity island SaPIbov2, a
163 mobile genetic element. Analysis of the *bap* flanking sequences revealed that *bap* is carried
164 by a transposon-like element. The transposon is inserted in the pathogenic island SaPIbov2
165 which is mobile without the presence of a helper phage (Penadés, 2006). The ability to
166 produce biofilms, associated with the presence of the *bap* gene has been shown to give
167 growth and persistence advantage to isolates from bovine chronic mastitis (Cucarella et al.,
168 2004). But, *S. aureus* is fully capable of forming biofilm in the absence of *bap* gene as shown
169 for some isolates (Vautor et al., 2006). These isolates were associated with the well-known
170 operon *icaADBC* (manuscript in preparation). As producing biofilm is an advantage for
171 virulence, it was the purpose of this study to look for the *bap* gene in *S. aureus* pathogenic
172 isolates. We propose two hypothesis, to be confirmed, explaining why the *bap* gene had not

173 spread amongst others *S. aureus* strains: i) the *bap* gene must have been acquired recently
174 by *S. aureus* in SaPIbov2 and consequently the gene has not been horizontally transferred
175 yet ii) horizontal gene transfer is not easy between different *S. aureus* lineages due to their
176 host specificities. The *S. aureus* lineages are supposed to be different between strains
177 recovered from different animal species. The Sau1 type restriction-modification system found
178 in *S. aureus* is one of specific mechanisms that controls the ability of mobile genetic element
179 to spread between strains (Waldron and Lindsay, 2006).

180 In conclusion, this study indicated that none isolates carried the *bap* gene suggesting
181 that the prevalence of this gene among *S. aureus* isolates should be very low. Finally, no
182 evidence of horizontal transfer of the *bap* gene between *S. aureus* recovered from different
183 animal species was found.

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