others. This observation is in line with many other S. aureus situations in which specific virulence factors are well correlated with an ex vivo or in vitro phenotype but are rarely linked to clinical epidemiology. We thus recommend being very cautious before using Sak as a predictive biomarker in clinic.

Note

Potential conflicts of interest. All authors: No potential conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Coralie Bouchiat1,2,4,5,8 Chloé Mehenni,6 Chloé Mehenni,6 Helène Meugnier,7,2,4,5,8 Michele Bes,1,2,4,5,8 Anne Tristan,1,2,4,5,8 and François Vandenesch1,2,4,5,8
1CIRI, International Center for Infectiology Research, Université de Lyon, 2Inserm, U1111, 3Ecole Normale Supérieure de Lyon, 4Université Lyon 1, 5CNRS, UMR5308, and 6Hospices Civils de Lyon, Centre National de Référence des Staphylocoques, France

References


Received 12 February 2014; accepted 8 April 2014; electronically published 1 May 2014.


Correspondence: Coralie Bouchiat, PharmD, Centre National de Référence des Staphylocoques, Laboratoire de Bactériologie, Centre de Biologie Est, 59 Blvd Louis Pinel, 69677 Bron Cedex, France (coralie.bouchiat@chu-lyon.fr).

The Journal of Infectious Diseases 2014;210:1341–3 © The Author 2014. Published by Oxford University Press on behalf of the Infectious Diseases Society of America. All rights reserved. For Permissions, please e-mail: journals.permissions@oup.com.
DOI: 10.1093/infdis/jiu246

Reply to Bouchiat et al

TO THE EDITOR—In our recent article on the role of staphylokinase (Sak) in skin infection [1], we made 3 observations: (1) Sak is involved in penetration into skin and establishment of new infectious foci; (2) lesions caused by a Sak-secreting strain are smaller in immunosuppressed individuals; (3) and Sak secretion is associated with uncomplicated superficial infections rather than with invasive, deep-seated cases. This last observation gained the attention of Bouchiat et al [2].

Identifying the role of Sak in staphylococcal virulence is a troublesome task. Previous findings pointed to better outcome of sepsis caused by Sak-secreting Staphylococcus aureus strains [3] and to decreased virulence of Sak-secreting strains in a murine model of S. aureus sepsis [4]. Our recent data, associating Sak secretion with noninvasive infections, fit into those previous observations. However, it is clear that such studies based on clinical isolate collections have their limitations. First, one needs to distinguish between the mere presence of the sak gene, the detectable secretion in vitro (when different secretion levels might be observed), and secretion during infection in vivo. Unfortunately, the third amount is pure speculation based on in vitro data. Another difficulty is caused by the presence of the sak gene on a prophage that also carries other virulence factors [5]. Therefore, the observed association of Sak with some disease outcome might be confounded by the effect of those other virulence factors. Finally, virulence of S. aureus is always multifactorial, caused by interaction of many virulence determinants; therefore, to detect any significant association, very large isolate collections need to be screened. Because of all those difficulties, we believe that only a combination of many independent observations can lead to solid knowledge. Therefore, we are very happy that Bouchiat et al [2] performed such an analysis on their collection.

In their analysis, Bouchiat et al [2] found no association of Sak (neither presence of the gene nor secreted levels of protein) with invasiveness of S. aureus. Instead, they observed a very clear association of Sak secretion with different clonal complexes of S. aureus (a detailed genetic analysis of S. aureus from our collection is unfortunately lacking, but we observed association of agr types with Sak secretion, confirming a relation of Sak secretion with the genetic background [unpublished data]). Therefore, they suggest that the difference observed in our collection might be due to a different presence of clonal complexes in our samples. This might indeed be the case. Unlike the association with strains from primary skin infection also reported in our article, the association of Sak with noninvasive strains was relatively weak. The association also only partly corresponded to the picture seen in our animal experiments, where Sak secretion affected local severity in skin in immunocompromised animals but had no impact on systemic spread. Therefore, the explanation of Bouchiat et al seems plausible, and at the current stage we have to suspect that Sak is not directly involved in prevention of staphylococcal invasiveness.

This case clearly illustrates how important it is to study different collections from all over the world and to reproduce all important findings. However, it also points to potential difficulties in analyzing Sak secretion in isolate collections. For example, we noted that the secretion data obtained by Bouchiat et al [2] differ strikingly from data we normally see in various isolate collections. In our essay, we always see 4 distinct groups of isolates: (1) a negative group (negative at polymerase chain reaction analysis and negative at chromogenic substrate assay even when the assay is performed during a prolonged overnight time); (2) a low-secreting group with very low SAK secretion (appearing negative in “regular” 1-hour chromogenic assay and positive only after prolonged overnight incubation);
(3) a group with moderate Sak secretion (moderately positive at regular chromogenic assay), and (4) isolates with extremely high Sak secretion (perhaps due to *sarA* or *sigB* deficiencies). It is, therefore, surprising that the characteristic “very high secreting” group is completely absent in the collection studied by Buchiat et al and that there is no clear division between isolates in respect to quantities of secreted Sak.

Color development in the chromogenic Sak activity assay should always be measured continuously. This allows one to differentiate between a real Sak activity (manifesting as continuous increase in absorbance) and random noise. More importantly, it allows one to choose a correct time point for comparison. If too many samples reach the maximum absorbance plateau, the comparison of values among the strains will be pointless. In our laboratory, the 1-hour time proved optimal, but this might differ among different experimental conditions. These issues urge us to carefully compare the methods used in different laboratories and develop one, easily reproducible method, which would yield similar results in laboratories all over the world.

**Note**

**Potential conflicts of interest.** All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Jakub Kwiecinski,1 Gunnar Jacobsson,2 Elisabet Josefsson,1 and Tao Jin1

1Department of Rheumatology and Inflammation Research, Institute of Medicine, Sahlgrenska Academy at University of Gothenburg, and 2Department of Infectious Diseases, Skaraborg Hospital, Skövde, Sweden

**References**


Received 8 April 2014; accepted 8 April 2014; electronically published 1 May 2014.

Correspondence: Jakub Kwiecinski, PhD, Department of Rheumatology and Inflammation Research, Institute of Medicine, Sahlgrenska Academy at University of Gothenburg; Box 490, 405-30 Gothenburg, Sweden (jakub.kwiecinski@rheuma.gu.se; jkwiecinski@gmail.com).

The Journal of Infectious Diseases 2014;210:1343–4
© The Author 2014. Published by Oxford University Press on behalf of the Infectious Diseases Society of America. All rights reserved. For Permissions, please e-mail: journals.permissions@oup.com.

DOI: 10.1093/infdis/jiu247