Quantification by Real-Time PCR Assay of *Staphylococcus aureus* Load: a Useful Tool for Rapidly Identifying Persistent Nasal Carriers

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The Cepheid Xpert MRSA/SA nasal PCR assay was compared to culture for quantifying *Staphylococcus aureus* load from 104 nasal samples (*r* = 0.91, *P* < 0.0001). Using a bacterial load-based algorithm, the test was found able to predict the carrier state in 32 of 35 healthy volunteers (22 persistent and 13 nonpersistent carriers).

Staphylococcus aureus nasal carriage is a well-recognized risk factor of *S. aureus* infection (11), and it is admitted that persistent nasal carriers of *S. aureus* exhibit a higher nasal bacterial load than intermittent carriers or noncarriers (7) and have a higher risk of infection (6). Accordingly, the rapid identification of persistent carriers with the aim of implementing a decolonization procedure could be very useful, notably before surgery or other at-risk procedures. However, the definition of *S. aureus* carrier status requires theoretically at least five consecutive nasal sampling episodes (11), which impairs considerably the feasibility of this determination in clinical practice. Recently, using a cohort of healthy volunteers, we proposed an algorithm based on one or two nasal sampling episodes for identifying *S. aureus* persistent nasal carriers (10). However, this characterization, based on the quantitative culture of nasal samples on a chromogenic medium, is time-consuming (24 to 48 h of bacterial growth) and labor-intensive.

Real-time PCR (rtPCR) assays demonstrated excellent specificity and sensibility for detecting *S. aureus* in nasal specimens (1). The Xpert MRSA/SA nasal assay, an rtPCR test including the extraction step, was shown able to reduce the technical work burden and to provide results in less than 2 h (5). The present study reports the ability of this assay, initially designed to provide qualitative results, to measure the nasal *S. aureus* load with the aim of quickly identifying persistent nasal carriers.

Statistical tests, including the *r* correlation coefficient and the area under the curve (AUC) of the receiver operating characteristic (ROC) curve, were performed using MedCalc (Mariakerke, Belgium) software, version 11.6. *P* values below the 5% level were considered statistically significant.

Foremost, the ability of the Xpert MRSA/SA nasal PCR assay to determine bacterial loads was assessed using 28 calibrated *S. aureus* specimens (strains NCTC 8325 and ATCC 43866) ranging from 10 to 10^9 CFU/ml. Quantitative cultures of these specimens were performed on duplicated blood agar plates using 10-fold serial dilutions in sterile water. The Xpert MRSA/SA nasal PCR assay was performed with the GeneXpert system (Cepheid, Sunnyvale, CA), by following the manufacturer's recommendations. A 200-µl volume of specimens was added to the S buffer and mixed thoroughly before being transferred into the cartridge's S well. The values for the crossing threshold (CT) were recorded from the GeneXpert software. CT values of calibrated specimens were highly correlated to the quantification obtained by culture,

with an *r* coefficient of 0.99 (95% confidence interval [CI], 0.97 to 0.99; *P* < 0.0001) and an equation of the regression line as log CFU/ml = (CT − 38.6)/(−2.93).

Then, the Xpert MRSA/SA nasal PCR assay was performed on 104 nasal samples collected during a previous study from 35 *S. aureus* nasal carriers (10). Briefly, flocked swabs (reference 552C; Copan, Brescia, Italy) were vortexed into 1 ml of phosphate-buffered saline (PBS), and 50 µl of the solution was plated onto a chromogenic medium (BBL CHROMagar Staph aureus; Becton, Dickinson). The *S. aureus* load was determined by counting the pink colonies. The identification of isolates at the species level was confirmed by matrix-assisted laser desorption ionization–time of flight mass spectrometry (Microflex; Bruker Daltonics, Bremen, Germany). From these 104 nasal samples, the equation above was used to convert CT values into bacterial loads. The *S. aureus* load deducted from the CT obtained by rtPCR was shown to be very close to that observed by culture, with an *r* coefficient of 0.91 (95% CI of 0.87 to 0.94; *P* < 0.0001) (Fig. 1).

In addition, the Xpert MRSA/SA nasal PCR assay was evaluated for its ability to segregate persistent and nonpersistent nasal carriers in a cohort of 35 *S. aureus* carriers, including 22 persistent and 13 intermittent carriers as defined by seven consecutive samples (10). The first nasal sample of the 35 carriers was quantified using the above-described equation. According to the algorithm retrieved from reference 10, for those patients with a bacterial load greater than 10^2 CFU/ml and less than or equal to 10^3 CFU/ml in the first swab, the second swab was quantified similarly (Fig. 2). With reference to the carriage status, using seven consecutive samples, the rtPCR-based algorithm was found able to predict the carrier state in 32 of 35 healthy volunteers. Three carriers, including two persistent and one intermittent, were misclassified by rtPCR, whereas one persistent carrier was misclassified by culture. Thus, close performances were observed for both techniques with
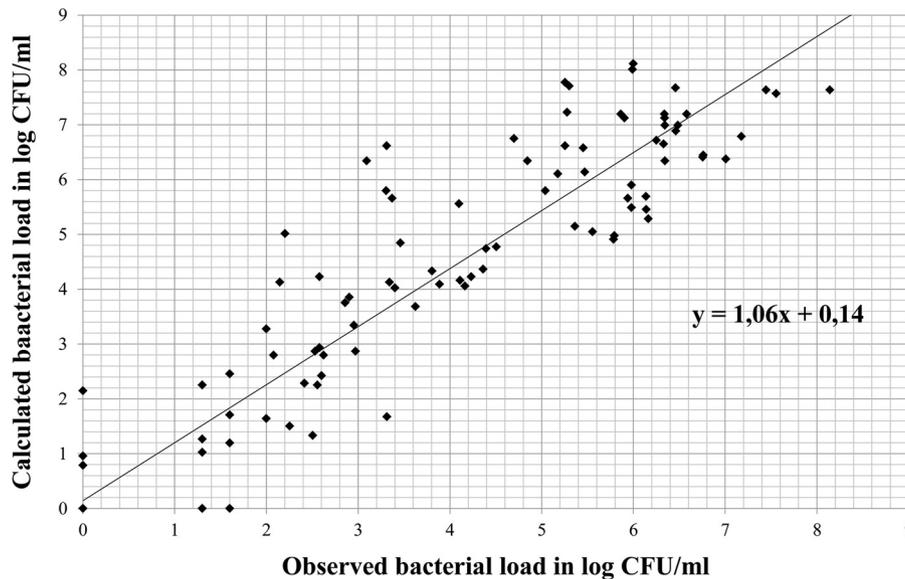
FIG 1 Correlation diagram from 104 nasal specimens between *Staphylococcus aureus* loads observed in bacterial culture (horizontal axis) and those extrapolated as described in the text from the CT values obtained with the Xpert MRSA/SA nasal PCR assay (vertical axis). The solid line corresponds to the correlation line, whose equation is on the scheme. The *r* coefficient was 0.91 (95% CI of 0.87 to 0.94), with a *P* value of <0.0001.

AUCs of 0.98 (95% CI, 0.86 to 1.00) and 0.92 (95% CI, 0.77 to 0.98) for the algorithm based on culture and rtPCR, respectively.

rtPCR assays are more and more used to provide quantitative results in different fields of bacteriological diagnosis (2, 4, 8), including in nasal samples (9). However, the Xpert MRSA/SA nasal PCR assay has been approved by the FDA and CE IVD (*in vitro* diagnosis) only for generating qualitative data. To our knowledge, this study is the first to demonstrate that this assay can provide a reliable quantification of the *S. aureus* nasal load. The higher *r* value obtained from calibrated specimens than from nasal sam-

ples could be explained by the fact that nasal samples are much more complex due to the presence of many other bacterial species that contribute to impairing the reproducibility of the culture testing.

In further experiments, the Xpert MRSA/SA nasal PCR assay was found able to discriminate accurately between persistent and nonpersistent nasal carriers using an algorithm based on one or two sampling episodes (Fig. 2). Interestingly, in the majority of subjects, the determination of the carrier state could be achieved with a single nasal swab, a situation currently observed in clinical practice. Also noteworthy is the fact that this test is easy to perform, requires no special training in molecular biology because the whole test is automated, including the extraction step, and can be completed in less than 1 h. Additionally, this test was designed to target the species-specific *spa* gene that is present in all the strains of *S. aureus*, whatever their geographical origin or their susceptibility to antibiotics, with the exception of rare *spa*-deficient strains (3).

The evaluation of this test was performed in a small-sized healthy adult population, and it would need to be tested further on a larger scale in patients susceptible to being targeted by a specific decolonization strategy (e.g., surgery, hemodialysis) and originating from different geographical origins. Despite these limitations, our results suggest that the Xpert MRSA/SA nasal PCR assay constitutes a very good candidate for identifying persistent carriers in clinical and epidemiological research studies of *S. aureus* colonization. In addition, this test could be useful for conducting prospective studies aimed to evaluate the correlation between bacterial load and *S. aureus* infection during at-risk procedures in persistent nasal carriers.
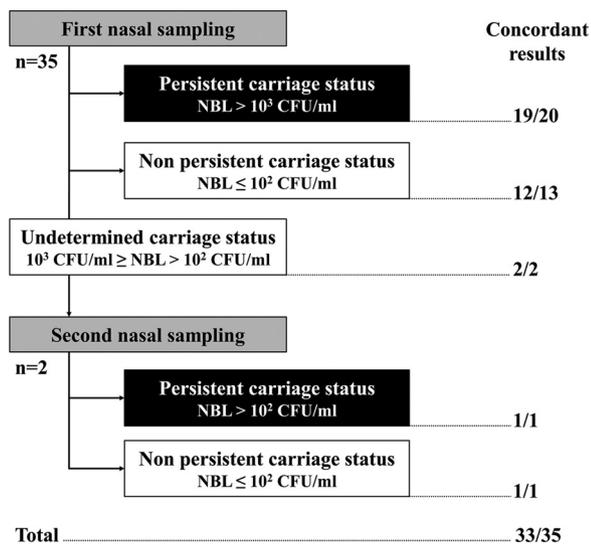
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FIG 2 Prediction of the *Staphylococcus aureus* carriage status (persistent or nonpersistent) in 35 healthy volunteers exhibiting *Staphylococcus aureus* in the nose according to the algorithm described in reference 10 by using Xpert MRSA/SA nasal PCR assay or culture (left). The numbers of concordant results by both techniques for each step of the algorithm are indicated on the right part of the diagram. NBL, nasal bacterial load.

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