An algorithm based on one or two nasal samples is accurate to identify persistent nasal carriers of *Staphylococcus aureus*

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Abstract

Persistent *Staphylococcus aureus* nasal carriers are at high risk of *S. aureus* infection. The present study delineates a simple strategy aimed at identifying rapidly and accurately this subset of subjects for clinical or epidemiological purposes. Ninety healthy volunteers were each identified as persistent, intermittent or non-nasal carriers of *S. aureus* by using seven specimens sampled over a 5-week period. By reference to this so-called reference standard, six other strategies aimed at simplifying and speeding the identification of persistent carriers and based on the qualitative or quantitative detection of *S. aureus* in one to three nasal samples were evaluated by the measure of the area under the curve of receiver operating characteristic diagrams. Among strategies using qualitative results, there was no statistical difference between protocols using seven and three samples. A threshold of 10^3 CFU of *S. aureus* per swab was found capable of defining persistent nasal carriage with a sensitivity of 83.1% and a specificity of 95.6%. These figures reached 95.5% and 94.9%, respectively, by using an algorithm including one or two nasal specimens according to the threshold of 10^3 CFU of *S. aureus* in the first swab. The latter two strategies were shown to be costly equivalents. The proposed algorithm-based strategy proved to be relevant to identify properly and consistently persistent nasal carriers of *S. aureus.* However, as it was built from data of healthy volunteers, it needs to be confirmed prospectively on patients potentially at risk for *S. aureus* infection.

Keywords: Carrier state, nasal mucosa, persistent carriage, risk factor, screening test, *Staphylococcus aureus*
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Introduction

The vestibulum nasi is considered the main site of *Staphylococcus aureus* colonization in humans (for a recent review see ref. [1]). Previous studies identified three patterns including persistent, intermittent and non-carrier states in approximately 20%, 30% and 50% of the whole population, respectively [1]. Persistent nasal carriers of *S. aureus* can be distinguished from intermittent carriers by a lower exchange rate of *S. aureus* strains or clones in repeated cultures [2–4] and by a higher mean number of CFU of *S. aureus* in the nose [1,4].

Patients with persistent carriage have been shown to exhibit a higher risk of *S. aureus* infection than patients with other statuses [5–7], at least in part by inducing a higher dispersion of *S. aureus* in the environment [1,4,5]. It has been shown recently that a decolonization procedure using mupirocin and chlorhexidine before surgery enabled a significant rate decrease of *S. aureus* infection [8]. Targeting patients who would benefit from such a drug-based prevention protocol could minimize the risk of emergence of *S. aureus* resistance [9,10]. Consequently, simple though fully reliable screening methods are needed to rapidly identify persistent nasal carriers.

However, the characterization of persistent carriage varies from one study to another and there is no consensual definition of the status of *S. aureus* persistent carrier. In previous

studies, this status was determined by using from 5 to 12 consecutive specimens taken over several weeks to months with a positive rate of at least 80% [4,6,11,12]. Seven successive nasal swab cultures was shown to reliably distinguish non-carriers from intermittent carriers [1,13]. Only one study proposed a 'culture rule' combining qualitative and quantitative results from only two nasal swabs taken within a 1-week interval to predict the persistent *S. aureus* carriage state with a positive predictive value of 79% [13].

The present study involving healthy volunteers was designed to simplify the accurate identification of *S. aureus* persistent carriers. By comparison to a reference protocol using seven successive specimens, a simplified algorithm using quantitative data from one or two nasal swabs in a short interval of time was found to be suitable to accurately segregate persistent carriers from subjects exhibiting a different status.

Methods

Study population

Ninety-three volunteers from the University Hospital of Saint-Etienne, France, were included in the study from March to April 2010. Volunteers were healthy healthcare workers, aged 18–65 years, and exhibited none of the following exclusion criteria: acute infection, chronic skin disease, current use of antibiotics and pregnancy. A written informed consent was obtained from all the volunteers. The study received the approval of the regional ethical research committee ('Comité de Protection des Personnes Sud-Est I').

Screening

Seven sampling episodes were scheduled to screen *S. aureus* nasal carriage over a 5-week period. During sampling episodes, nostrils were sampled independently with nylon flocked swabs (Regular nylon flocked swab 552C, microRheologics, Brescia, Italy) and rayon swabs (Fastidious anaerobe swab 108C, Copan, Italy) randomized according to the nostril side [14]. The sampling episodes were scheduled every 2 days for the first three specimens (D0, D2, D4) and once a week for the last four specimens (D7, D15, D23, D31). Samples were taken by one trained researcher following a pre-defined protocol; swabs were wetted in physiological serum, inserted approximately 2 cm into the anterior nostril and rotated five times [14].

Microbiological procedures

Samples were processed within 2 h. Swabs were vortexed for 10 s in 1 mL phosphate-buffered saline and 50 μL of this solution was plated onto an appropriate chromogenic medium (BBL™ CHROMagar™ Staph aureus, Becton Dickinson, Le Pont de Clair, France). Plates were read after 24 h and 48 h of growth at 36°C and pink colonies were plated onto blood agar for identification by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Bremen, Germany) [15–17]. The bacterial load, expressed as CFU/swab, was determined by the number of pink colonies after 48 h of growth on the chromogenic medium. The limit of detection was 20 CFU/swab of *S. aureus* and adapted dilutions were performed to quantify high bacterial loads. As the two nostrils were screened independently and in a randomized way, the highest bacterial load was selected for analysis.

Gentoyping of *S. aureus* isolates

The strains of *S. aureus* were characterized by arbitrarily primed PCR (AP-PCR) using the primers ERIC2 and I referred to in ref. [18], as previously described with minor changes [19]. Briefly, the DNA extraction was performed in a QIAsymphony® machine using a virus/bacteria mini kit (Qiagen, Courtaboeuf, France). The amplification step was performed in a GeneAmp® PCR System 9700 thermocycler (Applied Biosystems, Villebon-sur-Yvette, France). The amplicons were stained with GelRed™ (Interchim, Montluçon, France) and separated in a 1.5% agarose gel (UltraPure™ Agarose, Invitrogen, Cergy Pontoise, France). The AP-PCR profiles were analysed with Quantity One Software (Bio-Rad, Marnes-la-Coquette, France). Strains were considered different if the profiles exhibited more than two bands of difference with at least one of two primers [19]. The susceptibility of each clone to methicillin was studied by detection of the *mecA* gene.

Definition of nasal carrier state

Assuming that persistent carriers are defined by a positive rate of nasal swabs of at least 80% [6,11,12,20], persons with six or seven cultures positive for *S. aureus* were classified as persistent carriers; those with negative results of all cultures were classified as non-carriers. All other persons were classified as intermittent carriers. This strategy was labelled the 'reference protocol'.

Evaluation of screening strategies of persistent *S. aureus* nasal carriage

Based on previously published protocols looking for nasal carriage of *S. aureus* [8,13,21–23], six different screening strategies, lettered from A to F, were evaluated. The first three strategies were based only on qualitative results of one (A), two (B) or three (C) consecutive positive samples. Strategies D and E relied on a single sample with a bacterial