Patient nostril microbial flora: individual-dependency and diversity precluding prediction of *Staphylococcus aureus* acquisition

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Abstract

The potential role of a patient's resident microbial flora in the risk of acquiring multiresistant bacteria (MRB) during hospitalization is unclear. We investigated this role by cross-sectional study of 103 patients at risk of acquisition of *Staphylococcus aureus* (SA), resistant (MRSA) or not (MSSA) to methicillin, recruited in four French hospitals. The flora was analysed by an exhaustive culture-based approach combined with molecular and/or mass-spectrometry-based identification, and SA strain typing. Forty-three of the 53 SA-negative patients at entry were followed for up to 52 weeks: 19 (44.2%) remained negative for SA and 24 (55.8%) became positive, including 19 (79%) who acquired an MSSA, four (17%) who acquired an MRSA and one who acquired both (4%). Fifty-one different species were identified among the 103 patients, of which two, *Corynebacterium accolens* and *Staphylococcus haemolyticus* ($p = 0.02$-0.01), were more prevalent in the absence of SA. However, the same number of patients carrying or not these two species acquired an MSSA/MRSA during follow-up, regardless of antibiotic treatment received. Clustering analysis showed that the microbial flora was highly specific to each patient, and not predictive for acquisition of MSSA/MRSA or not. Patient-specific microbial resident flora is not predictive of SA acquisition.

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Introduction

Antimicrobial resistance has become a central challenge in the control of infectious diseases, especially in hospitals [1,2]. Strategies to prevent nosocomial infection will be more effective if they are guided by a comprehensive knowledge of risk factors determining the acquisition of these agents by the hospitalized patient. Such knowledge is especially pertinent when the same agents also exist in the community [3] or are widespread in some populations [3], rendering sourcing of the patient colonization more difficult.

Important risks factors, such as advanced age, underlying diseases and severity of illness, and inter-institutional transfer of patients proved to be common and universal whatever the agents [4]. However, the potential natural protection developed by hosts might also play an important role, by limiting the success of colonization by MRB in their own ecosystem. This question still needs to be addressed: studies examining in particular the role of host factors in SA acquisition are still scarce, especially with regards to SA nasal carriage [5,6]. Two main mechanisms are putatively involved: host factors or immunity [7–9], whether

acquired [10] or innate [11], and the barrier effect of the microbial resident flora (also termed bacterial interference) [12–16].

Uncertainties still persist regarding a potential barrier effect of the resident nostril microbial flora in preventing SA acquisition, despite the identification of several genus/species potentially involved [12–16]. The barrier effect of these genus/species during follow-up of naïve SA patients is still not known. In addition, published studies mostly involved healthy subjects only, rarely patients [16]. No longitudinal studies had been yet conducted on a hospitalized patient population who are also at risk of SA acquisition.

Here, we investigated the potential role of the resident nostril microbial flora in SA acquisition, by analysing the nostril flora of 103 handicapped patients with neurological disorders. These patients, hospitalized for a long period of time in four rehabilitation centres, often suffered from multiple infectious complications and, as a consequence, received many broad-spectrum antibiotics, providing a favourable environment for detecting stochastic acquisition of MSSA/MRSA and parameters involved. We undertook a systematic quantification of any isolated bacteria from a nasal swab taken from patients at inclusion and during the follow-up for patients' naïve for SA colonization at inclusion.

Materials and Methods

Epidemiological study

Our study was performed from January 2008 to December 2010 as part of a clinical research programme named ASAR (detailed in Supplementary Data) in four French hospitals. Patients were asked and gave full consent to participate in the ASAR project. The first 103 recruited patients screened for nasal SA before inclusion, had their nasal swab also used for analysis of their microbial flora. This group comprised 50 patients that were positive and 53 patients that were naïve for SA carriage at entry. The 53 SA-naïve patients were then followed-up for 13 weeks if they did not acquire an SA and 52 weeks in the case of SA acquisition (Supplementary Figure 1).

Nasal swab collection and culture for SA detection

Alginate swabs, soaked beforehand in sterile physiological saline solution, were rotated five times around the inside of both nostrils while applying constant pressure. Swabs were then placed in Stuart's transport medium (500 μl, Transwab, Medical Wire and Equipment, Corsham, Wiltshire, England) and kept at room temperature until arrival at the Microbiology Laboratory (Raymond Poincaré Hospital, Garches, France).

A 100-μl aliquot was plated on selective and non-selective media for MSSA/MRSA isolation as detailed in Supplementary Data. The rest of the heavy dispersed suspension was then stored at −80°C for further use. Screening for MRSA and antimicrobial susceptibility testing were performed as detailed in Supplementary Data.

Culture and quantification of the microbial flora

One hundred-microlitre aliquots were serially diluted up to 10^{-4} and plated on different selective and non-selective media (resulting in 16 conditions per patient, see supplementary figure 2), and incubated at 37°C in aerobic ± 5% CO_2 and anaerobic conditions. Plates were read at 24 h and 48 h after incubation (see Supplementary Data).

MALDI-TOF and molecular species identification of isolated bacteria

Matrix-assisted laser desorption/ionisation-time-of-flight mass spectrometry (MALDI-TOF) and/or DNA sequencing were used for specific identification as previously described [17,18].

Spa-typing of SA isolates

Spa typing was performed as previously described [19] and is summarized in Supplementary Data.

Clustering-based analyses of composition of nostril flora

For each patient, each of the species isolated from the microbial nostril flora, or of the 16 most represented species, was coded 1 when present or 0 when absent. The resulting flora composition fingerprints were then used in the Bionumerics 6.5 software (Applied Maths) as strings of categorical characters to construct minimum spanning trees, in order to analyse the clustering of SA and non-SA carriers according to the flora composition.

Statistical analysis

T-tests for independent samples, using R software, and logistic regression, using Stata software (Data analysis and Statistical Software, TX, USA), were utilized in univariate and multivariate models to evaluate probability for differences between patients who were SA carriers or non-carriers (see Supplementary Data); $p \leq 0.05$ was considered statistically significant.

Results

Quantitative comparison of the SA+ and SA− patient population

The 103 patients included in the study were all disabled, requiring extensive medical and nursing care, leading to bacterial over-exposure (Table 1). Patients had bedsores and close regular contacts with nursing and physiotherapy staff, and