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Title: Is nasal carriage of Staphylococcus aureus the main acquisition pathway for surgical-site infection in orthopaedic surgery?

Article Type: Article

Keywords: Staphylococcus aureus; nasal carriage; carrier; surgical site infection; hospital infection; orthopaedic surgery

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Abstract: Purpose
The endogenous or exogenous origin of Staphylococcus aureus, responsible for orthopaedic surgical site infections (SSI), remains debated.
Methods
We conducted a multicentre prospective cohort study to analyse the respective part of exogenous contamination and endogenous self-inoculation by S. aureus during elective orthopaedic surgery. The nose of each consecutive patient was sampled before surgery. Strains of S. aureus isolated from the nose and the wound, in case of SSI, were compared by antibiotypes or pulsed field gel electrophoresis (PFGE).
Results
3908 consecutive patients undergoing orthopaedic surgery were included. Seventy seven patients developed an SSI (2%), including 22 related to S. aureus (0.6%). S. aureus was isolated from the nose of 790 patients (20.2%) at the time of surgery. In multivariate analysis, S. aureus nasal carriage was found as a risk factor for S. aureus SSI in orthopaedic surgery. However, only 9 subjects exhibiting S. aureus SSI had been found carriers before surgery: when compared, 3 pairs of strains were considered different and 6 similar.
Conclusion
In most cases of S. aureus SSI, either an endogenous origin could not be demonstrated, or pre-operative nasal colonisation retrieved a strain different from the one recovered from the surgical site

Response to Reviewers: Reply to reviewers

Thank you for these interesting comments. Please find below our answers to the queries of the two reviewers.

Reviewer 1

It is unfortunate that they did not screen other sites such as the throat and perineum.

We agree with this comment. Initially, the protocol included a rectal swabbing but many patients were reluctant to this proposal. Due to a high rate of missing data during the initial step of the study, we decided to stop the sampling of this site. The following sentence was added in the discussion section of the new manuscript (page 11; lines 12 to 14): "Our protocol included initially a rectal swabbing before surgery; however, since many patients denied this sample, the data regarding rectal specimens could not be taken into consideration". Concerning the throat sampling, when the study was designed in 2002, sampling this site was not recommended since this specimen was not considered at this time to increase significantly the sensitivity of the recovery of S. aureus from the upper respiratory tract as shown later by Metz et al. in 2007 [ref 23] (this point is discussed page 11, lines 14 to 17).

They could point out that sole carriage at these sites is rare (perhaps 10%) and add this to their estimates of the possible size of the problem (20% larger!). Nasal carriage rather than carriage should be in the text.

We also agree with this comment, which is emphasised in the new discussion section (page 11; lines 14 to 17): "With a single nasal sample and in the absence of additional throat and rectal specimens, the frequency of carriage of S. aureus in our cohort was certainly underestimated, as illustrated by the figure of 20.2% of nasal carriers in this study compared to 37.1% in the one of Mertz et al [23] that combined nasal and throat samplings."

Comment 1 about CNS data:
1) Table 3 has overall data. They could compare the antibiograms of the individual isolates and see if there are very different strains. I doubt the organisms are available for molecular analysis (if so this could be "future work" for the discussion) but at least the antibiograms could be compared by ward and centre and see if there are any similarities and differences and informed guesses made as to the likelihood of cross infection e.g. quinolone resistance is usually mutational
2) All the other infected organism data. Again what about antibiograms and possible cross infection?

The goal of the study was to address the potential link between S. aureus nasal carriage and occurrence of S. aureus SSI, and not to study CNS or other bacterial cross infections. Strains other than S. aureus were not kept for further analysis. By contrast, the potentiality of cross infections with S. aureus was investigated and presented with some details in the revised manuscript (see below).

3) S aureus data.
We need to see their rule set for deciding why the extra 5 strains were different or similar. This is a difficult area and must be explained.

The way used to compare antibiotypes is reported in the method section (page 6, lines 12 to 16). As required by the referee, additional information regarding the comparison of antibiotic profiles has been added in the footnotes of Table III.

They should compare the 13 SA from non carriers (and indeed the carriers) to see if there are possible issues of cross infection in terms of time person and place of the affected patients. Are these strains similar by PFGE? Are they similar to any of the carriers?

From the 13 S. aureus SSI strains isolated from non nasal carriers, 8 were available for comparison by PFGE: all these strains exhibited independent profiles, also different from those of strains isolated from the nose of colonised patients. The 5 remaining strains were isolated from non nasal carriers hospitalized in 5 different centres, excluding cross transmission. These data were added in the result section page 9 lines 10 to 13.

Table IV only has death related risk analysis not as stated on page 10 lines 23-24 for SSI and SA nasal carriage.

Because of the low number of SSI infections, the multivariate analysis was run with a maximum of 3 covariates selected among the most significant in the univariate analyses. This sentence was added in the method section (page 8 lines 8 to 10). We also modified the sentence presenting Table IV (page 10 lines 14 to 15).

4) There are also the therapeutic issues raised by the AST results for all categories of infections above. These are perhaps a little peripheral but I think it would add to the paper or perhaps be another one if there are clinical outcome data?

Data regarding therapeutic issues are not presented in this paper. Another clinical paper focusing on these results is in preparation

Other points
How were nasal swabs taken: were the swabs moistened?

The precision regarding moistened transport medium was added in the method section (page 5, line 1).

Was the PFGE protocol validated to be able to distinguish strains and how?

The criteria for PFGE interpretation are described page 6 lines 10 to 12 "For a same patient, according to Tenover's criteria [18], two S. aureus strains were classified as epidemiologically distinct if a difference of more than three bands was ascertained between the PFGE profiles performed in a same run".

Reviewer 2

First this was a multicenter study and in these kind of studies cluster effects may occur. In the multivariate analysis, adjustment for cluster effects was not included. This would strengthen the conclusions.

As pointed by the reviewer it is important to look for cluster effect. This was investigated by adding a "centre" variable in the different statistical analyses. This variable did not remain in the final model used for multivariate analysis. But as required by the referee, we performed another multivariate analysis using windows SAS® 9.1 software to adjust for cluster effect. This was added in the method section (page 7, line 25). We replaced the results of the previous multivariate analysis performed using SPSS software by the results of this multivariate analysis adjusted for cluster effect (Table IV).

Second, the authors conclude that the majority of the S. aureus strains are of exogenous origin. They put it quite strong in the conclusions that 'at least 16 were not endogenous'. In my opinion this is not correct. The nasal cultures were negative but this may have been a false-negative result, e.g. due to the sampling technique. As the carriage rate was only 20% it is clear that the culture technique was not very sensitive (normally 30% of the population is nasal carrier). Therefore, I suggest to modify this conclusion.
In the majority of cases an endogenous origin could not be demonstrated would be more correct.

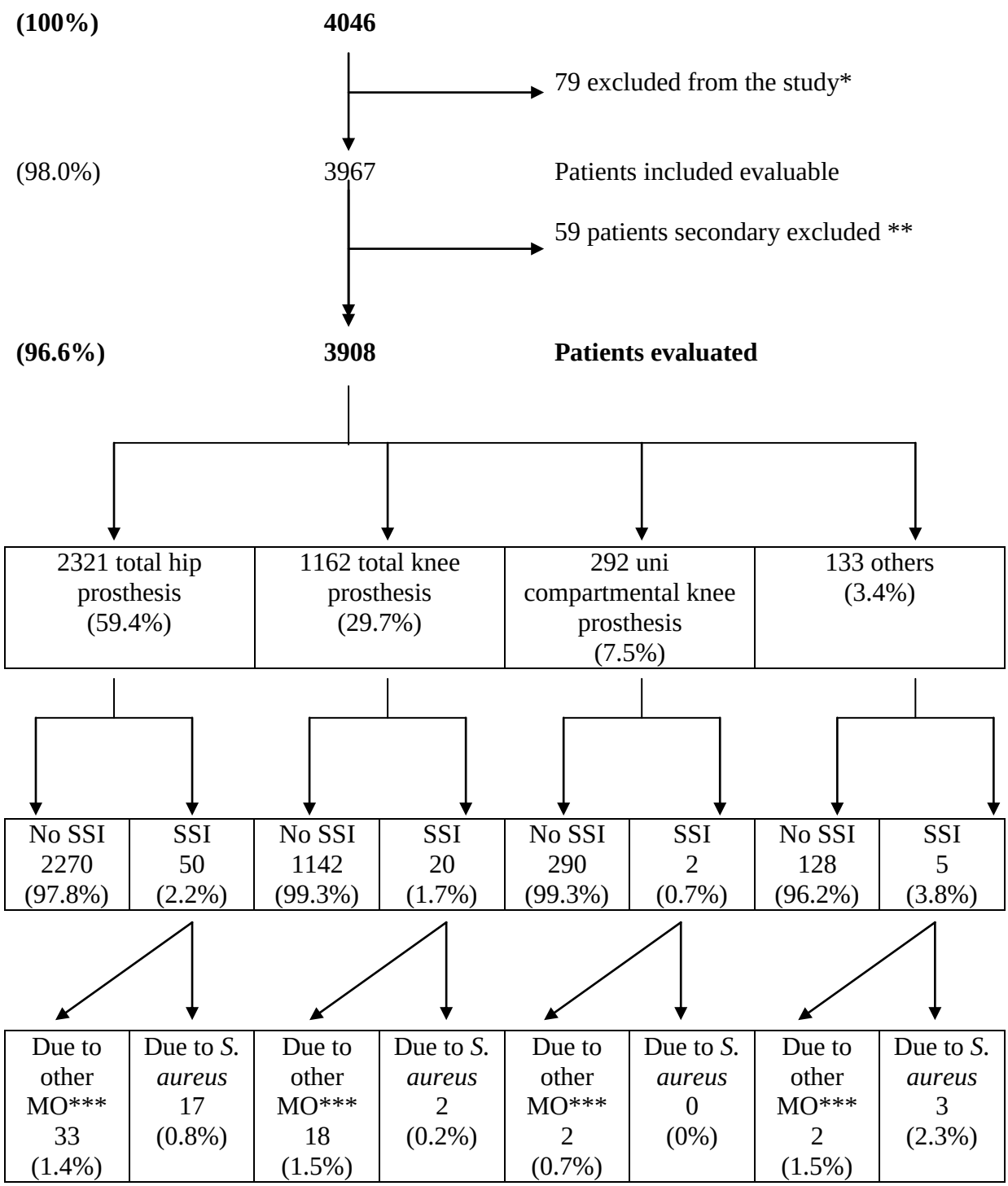
We agree with this comment. The discussion section was amended accordingly (see answer to reviewer 1 and additional sentence in page 11; lines 14 to 17)).
As suggested by the referee, we also modified the conclusion; the sentence "In most cases of S. aureus SSI, pre-operative nasal colonization was not documented or retrieved a different strain from the infecting pathogen" was replaced by the following one in the new manuscript (page 12, lines 20 to 22 and in the abstract): "In most cases of S. aureus SSI, either an endogenous origin could not be demonstrated, or pre-operative nasal colonisation retrieved a strain different from the one recovered from the surgical site"

Also, it would be important to provide information on the typing of the strains from non-carriers as well. I wonder whether there were many similar strains within centers suggesting a common source?

This point was also addressed by reviewer 1. No cross transmission of S. aureus was documented in this cohort. This important point was added in the result and discussion section, page 9 lines 10 to 13. The following sentence was added page 12 line 11 to 12 "It is noteworthy that, in our study, no cross transmission of S. aureus within centres was documented".

Figure

Figure 1:

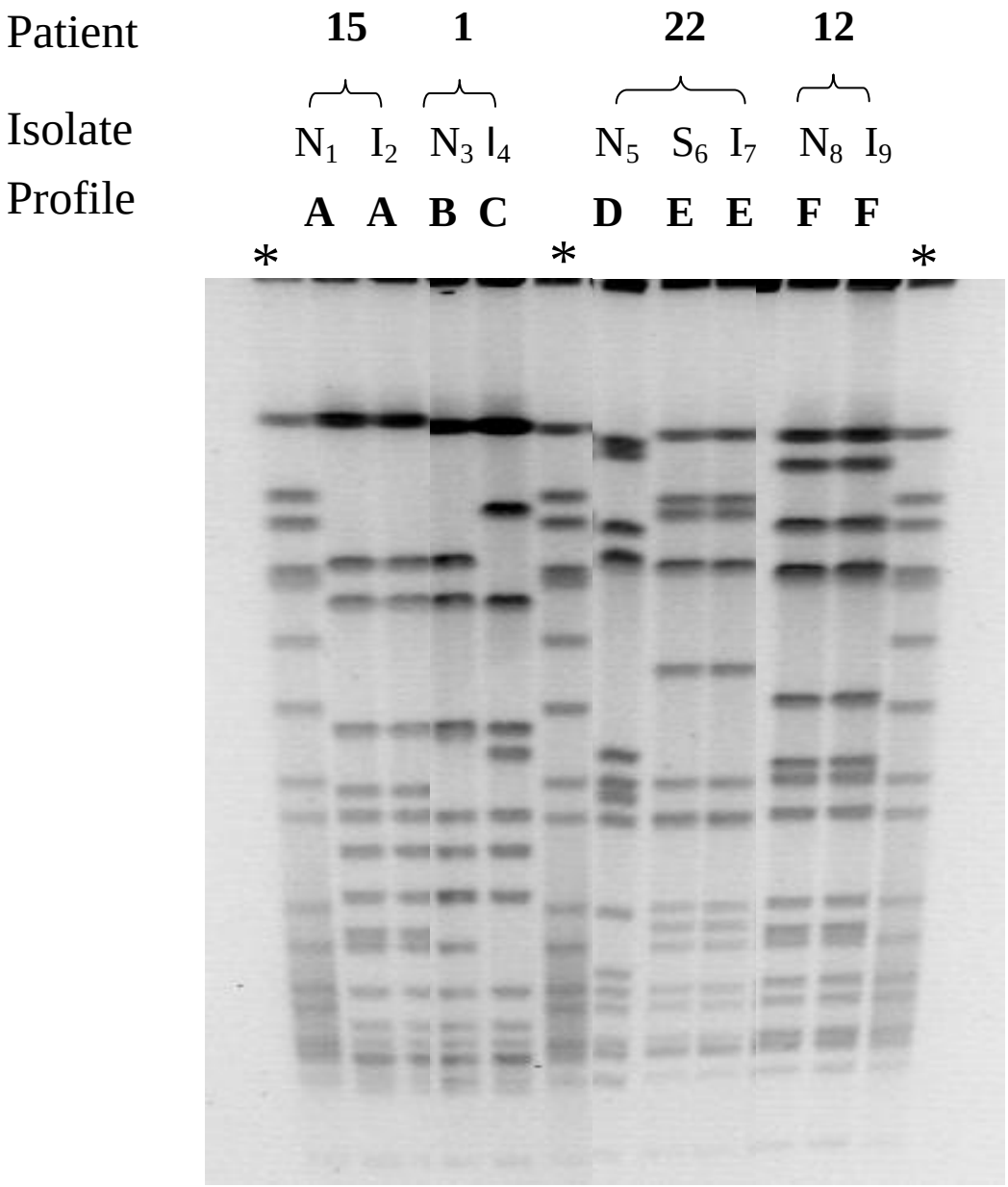


* 2 suspicions of prosthesis infection, 4 other foreign materials, 2 canceled surgeries, 1 other surgery, 1 hip prosthesis luxation, 1 without clinical data. The remaining patients had no nasal sampling

** no clinical data (n=18) or no nasal sampling (n=41)

*** MO: micro-organisms

Figure 2:


Patient
15
1
22
12
Isolate
N_1 I_2 N_3 I_4 N_5 S_6 I_7 N_8 I_9
Profile
A A B C D E E F F
*
*
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Conflict of interest statement

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Abstract

Purpose

The endogenous or exogenous origin of *Staphylococcus aureus*, responsible for orthopaedic surgical site infections (SSI), remains debated.

Methods

We conducted a multicentre prospective cohort study to analyse the respective part of exogenous contamination and endogenous self-inoculation by *S. aureus* during elective orthopaedic surgery. The nose of each consecutive patient was sampled before surgery. Strains of *S. aureus* isolated from the nose and the wound, in case of SSI, were compared by antibiotypes or pulsed field gel electrophoresis (PFGE).

Results

3908 consecutive patients undergoing orthopaedic surgery were included. Seventy seven patients developed an SSI (2%), including 22 related to *S. aureus* (0.6%). *S. aureus* was isolated from the nose of 790 patients (20.2%) at the time of surgery. In multivariate analysis, *S. aureus* nasal carriage was found as a risk factor for *S. aureus* SSI in orthopaedic surgery. However, only 9 subjects exhibiting *S. aureus* SSI had been found carriers before surgery: when compared, 3 pairs of strains were considered different and 6 similar.

Conclusion

In most cases of *S. aureus* SSI, either an endogenous origin could not be demonstrated, or pre-operative nasal colonisation retrieved a strain different from the one recovered from the surgical site

Keywords. *Staphylococcus aureus; nasal carriage; carrier; surgical site infection; hospital infection; orthopaedic surgery*

Trial registration. National Project of Hospital Clinical Research (PHRC) “DEPISTAPH” Ministère de la Santé France.

Introduction

The risk of prosthetic joint infection is less than 2 percent after joint replacement [1-3] or after implantation of internal fracture-fixation devices [4]. However, the large number of such procedures performed annually makes these infections highly significant in terms of mortality, morbidity and costs, as far as the elderly population is increasing in industrialized countries [5-7]. It is a well-known fact that *S. aureus* carriage predisposes to post-operative staphylococcal infection in general surgery [8], in heart surgery [9-10], as in orthopaedic surgery [11]. However, Kalmeijer *et al*. [11], in orthopaedic surgery, realized their study in only one centre, with a limited number of patients. Moreover, the studies showing a substantial reduction of SSI among patients receiving mupirocin were all compared to historical control [8, 12-14], whilst Kalmeijer *et al*.[15], in a double-blind, randomised, placebo-controlled study, in orthopaedic surgery, failed to show any beneficial effect. So, the exogenous pathway of contamination in orthopaedic surgery remains an important hypothesis and we undertook a large prospective multicenter cohort study, to determine whether the nasal carriage of *S. aureus* is the main pathway responsible for infection in orthopaedic SSI.

Patients and methods

We conducted a prospective epidemiological multicentre observational study to estimate the relative part of subjects with *S. aureus* nasal carriage in patients with SSI following prosthetic and internal fracture-fixation device surgery, and in non-infected patients. We compared, in SSI patients, the strains of *S. aureus* colonizing the anterior nares and that isolated from the site of infection.

Patients. All consecutive patients undergoing scheduled orthopaedic surgery with implantation of prosthesis or internal fracture-fixation device, in seventeen centres (see appendix), were included from June 2003 to January 2007. A control visit was performed by the surgeon at least one month and one year after surgery. Exclusion criteria were non-programmed surgery and suspicion of infection at the site of surgery.

A written informed consent was obtained for all patients. The study protocol was approved by the regional ethical research committee *(Comité de Protection des Personnes Rhône-Alpes I).*

The preparation of patients' skin included at least one preoperative shower with a soap containing either chlorhexidine or polyvidone iodine, cleansing of the skin with an antiseptic soap followed by a disinfection with an alcoholic antiseptic in the operative theatre. Antibiotic prophylaxis was done with cefazolin, 2 grams at the anaesthetic induction. Mupirocin nasal decontamination was not used whatever the results of the sampling.

One to three months before surgery, the study was explained to the patient during surgical consultation. Written information was delivered. The nose of the patient was sampled at the admission. To ensure performance and reproducibility of the nasal sampling, nurses were trained and a written protocol was available in each participating unit. The patient's practitioner was informed of the study and asked to refer the patient to the surgeon if a SSI was suspected. In the latter case, at least 3 bacteriological samples were taken at the site of the suspected infection either per-operatively (without antimicrobial prophylaxis) or by aseptic aspiration using ultrasound guidance. Direct examination and bacteriological culture were systematically performed.

Bacteriological methods

Nasal cultures: One swab was used to sample both nares and plated onto sheep blood agar plate or onto specific *S. aur*eus Chromastaph medium (Biomérieux, Marcy l'Etoile, France)

within 24 hours after sampling if a moistened transport medium was used (Transtube®, Medical Wire and Equipment Co., Corsham, England or Amies agar gel, Copan, Brescia, Italy) or within 2 hours in the opposite case. Plates were incubated up to 48h at 36°C. The identification of *S. aureus* isolates and their sensitivity to antibiotics were determined by standard microbiological procedures.

As for nasal samples, swabs were cultured within 2 hours after sampling if no transport medium was used, and within 24 hours in the opposite case. Fluid samples were examined by light microscopy after Gram staining. They were cultured both aerobically and anaerobically into blood culture bottles and monitored in an automated system. Solid samples were dissected into tiny pieces with a lancet in a safety cabinet and then ground down. The homogenate was cultured onto aerobic and anaerobic media. The media were incubated at 36°C for at least 48 hours for plate cultures and 15 days for liquid broths.

In addition to phenotypic characteristics, all the available couples of strains of *S. aureus* isolated from the nose and the infected surgical site were compared by PFGE using *Sma*I. The available strains were centralised in a single laboratory where PFGE was performed. Plugs containing DNA were prepared following a rapid lysis procedure [16]. Bacterial cells from an overnight broth culture were mixed with 1.6% of SeaPlaque agarose (Cambrex, Rockland, USA). The plugs were incubated in lysis buffer containing 250 IU of lysostaphin (AMBI Inc, Lawrence, NY, USA) for 2 hours at 37°C, followed by a one-hour incubation at 50°C with ESP buffer containing 100 mg/L of proteinase K. Plugs were rinsed with TE buffer, incubated in TE with PMFS for 30 min at 37°C before final washing in TE buffer. The genomic DNA inserts were digested at 25°C for 2 h with 20 UI of *Sma*I enzyme. The fragments were separated by PFGE with a CHEF-DrII apparatus (Biorad, Ivry sur Seine, France) in a 1.1% agarose gel (Gigaphor, Promega, Les Ullis, France) in TBE buffer, at 14°C under a field strength of 6 V/cm and a linear ramp of 1s- 28s for 21 hours.

Definitions

Nasal carriage of *S. aureus*:

Carriage was considered if a strain of *S. aureus* was recovered at one sampling. Patients without documented *S. aureus* carriage were classified non carriers.

SSI:

Definitions for SSI were those edited by the Centers for Disease Control (CDC) [17].

Classification of pairs of S. aureus strains isolated from the nose and the SSI in a same patient

For a same patient, according to Tenover's criteria [18], two *S. aureus* strains were classified as epidemiologically distinct if a difference of more than three bands was ascertained between the PFGE profiles performed in a same run. When the *S. aureus* strain isolated from the surgical site was not available for typing, the antimicrobial patterns were used to compare the strains isolated in a same patient: a difference in antimicrobial susceptibility of two or more molecules signed different strains; in case of difference concerning one or no antibiotic, strains were considered phenotypically similar.

Data recorded

The following variables were recorded for each patient: age, sex, Body Mass Index (BMI), tobacco use, underlying diseases (malignancy, diabetes, malnutrition, immunodeficiency, renal deficiency), medications (particularly antibiotics one month before surgery and immunosuppressive drugs as corticosteroids), rheumatoid arthritis, kind of procedure performed and type of prosthesis, cement use, antimicrobial prophylaxis, date of the admission to the hospital, date of surgery, date of discharge, dates of follow-up, class of wound contamination, American Society of Anaesthesiologist (ASA) score, duration of

surgery, prosthesis first implantation or revision, blood transfusion, hematologic diseases and notable blood collection after surgery. The data of nasal samplings and their results were also recorded. In case of SSI, the following data were collected: date of infection, localization of infection (superficial or deep), clinical symptoms, microbiological identification and treatment.

Outcomes

The primary outcome was to identify risk factors of SSI due to *S. aureus* by using multivariate analysis with special emphasis to the variable "nasal carriage of *S. aureus*". The secondary outcomes were (i) to evaluate the proportion of *S. aureus* strains isolated in a same patient from the nose and SSI found similar by microbiological methods, (ii) to estimate the frequency of *S. aureus* nasal carriage in our cohort of patients, and (iii) to estimate the risk of *S. aureus* SSI.

Statistical analyses

We estimated that SSI would occur in 1% of hip prosthesis surgery and in 2% of knee prosthesis surgery and that the proportion of *S. aureus* responsible for infection would be 40 %. Overall, we calculated that 4200 patients were needed for this study to detect a 20% difference of *S. aureus* nasal carriage between patients without and with SSI due to *S. aureus*, given a two-tailed alpha level of 5% and a statistical power of 80%. It was hypothesized that an attending number of 40 SSI due to *S. aureus* would give sufficient discriminative power to compare pairs of strains (nose and SSI) by molecular techniques.

The software used for the collection of the recorded data was Epi-info, version 6.04d Fr (CDC-WHO). The SPSS software, version 16.0 (Chicago, Illinois, USA) was used for univariate and multivariate analyses. Windows SAS® 9.1 software was used to adjust for

cluster effect in the multivariate analysis. The rates of SSI and SSI due to *S. aureus* were calculated globally and according to patients' nasal carriage of *S. aureus* and to the type of device implemented. These rates were also stratified according to the NNIS index. Fisher's exact test was used for analysis of categorical variables. The Wilcoxon non parametric test was used for mean comparisons. Two multivariate analyses were carried out to evaluate (i) risk factors for SSI and (ii) risk factors for SSI due to *S. aureus*. To adjust for confounding factors, variables with a *P* value below the 0.05 significance level in univariate analysis were entered into a multiple logistic regression model. Because of the low number of SSI infections, the multivariate analysis was run with a maximum of 3 covariates selected among the most significant in the univariate analyses. *P* values below the 5% level were considered statistically significant.

Results

Demographic and clinical data

The total duration of the study was 4 years (3 years of inclusion and one year of supplementary follow-up; end at January 2008). Four thousand and forty-six patients were included and 3908 (96.6%) patients were evaluated. Demographic characteristics of the patients are shown in Table I. The causes for exclusion (138 patients) are described in Figure 1. Seventy-seven patients (2%) were considered as victims of SSI. The overall infection rate was 2.2% for total hip replacement, 1.7% for knee replacement and 1.6% for partial prosthesis and fracture-fixation devices.

Microbiological data

The most common infecting micro-organisms were coagulase-negative *Staphylococcus* spp. [0.9% of patients], followed by *Staphylococcus aureus* [0.6%], and Gram negative bacteria [0.4%]. Of these infections, 61% arose in the first three months following surgery. There was no significant change in the infection rate or type of infecting micro-organism over the course of the study. As shown in Table II, 94 micro-organisms were found responsible for SSI. Fourteen SSI were polymicrobial.

Twenty-two SSI due to *S. aureus* were documented; 13 of these patients were classified as nasal non carriers and 9 as nasal carrier.

Within the strains isolated from the 13 patients classified as nasal non carriers, 8 were available for typing by PFGE. All of these strains exhibited independent profiles (data not shown). The 5 remaining strains were isolated from patients hospitalized in 5 different centres, excluding cross transmission.

In patients classified as nasal carriers, the 9 couples of strains isolated from SSI and nasal specimen were compared according to their genotype (4 cases) and/or their antimicrobial susceptibility (5 cases). From the 4 pairs of strains compared by PFGE, 2 pairs exhibited different profiles whereas 2 other pairs were shown to share a similar profile according to Tenover's criteria (Figure 2). All of these strains were different from those isolated from infected patients classified as non carriers (data not shown). For the 5 remaining cases, nasal samples were not available for typing in 2 cases and, in 3 cases, strains recovered from the surgical site were not stored because isolated in laboratories located outside an university hospital; when compared according to antibiotypes, one of these 5 pairs was classified different whereas the four others were classified similar because of identical or minor differences in antimicrobial susceptibility (see footnotes of Table III for details).

Overall, by combining the results of genotypic and phenotypic typing methods, from a total of 22 *S. aureus* SSI, no *S. aureus* nasal carriage was noted in 13 patients; in the 9 remaining patients, 3 pairs of strains were different and 6 pairs were similar (Table III). So, with the hypothesis of maximum bias, at least 16 of the 22 cases of *S. aureus* SSI were independent of nasal carriage of *S. aureus* at the time of surgery.

SSI and nasal carriage

S. aureus was isolated in the nose of 790 patients at the time of surgery (20.2%). Methicillin resistant *S. aureus* (MRSA) strains were isolated in only 0.6% of patients. The overall risk of SSI was measured at 2% (CI 95% [1.6 – 2.4], range 0 – 7.6 according to the centre). The prevalence of SSI due to *S. aureus* was 0.6 % (CI 95% [0.36 – 0.84]).

Risk factors for SSI

Univariate and multivariate analyses were performed to assess the risk factors for SSI (Table IV). In multivariate analysis, significant risk factors were tobacco use, wound haematoma and NNIS score for the occurrence of SSI., nasal carriage together with tobacco use and NNIS score for SSI due to *S. aureus* (Table IV).

Discussion

The present study is, to our knowledge, the largest evaluation of nasal carriage of *S. aureus* in patients undergoing orthopaedic surgery. It confirms that nasal carriage of *S. aureus* at the time of surgery is a risk factor for *S. aureus* SSI in orthopaedic surgery. However 13 out of 22 *S. aureus* SSI occurred in non carriers, and the majority (16/22) of *S. aureus* SSI were

independent of nasal carriage, suggesting an exogenous pathway of contamination. Recently, two studies performed in cardiothoracic surgery [19] and in general surgery [20] showed that endogenous nasal contamination was the major acquisition pathway for methicillin-susceptible *S. aureus* (MSSA), whereas exogenous acquisition pathway was essential for methicillin-resistant *S. aureus* (MRSA), underscoring the crucial role of hospital infection control measures.

Several criticisms can be addressed to our study. First, we have limited the sampling to the anterior nares since this site is considered to be the primary colonization site of S. aureus [21, 22]. Recently [23], a large study has highlighted the importance of the throat as a significant site of S. aureus carriage, with an additional sensitivity of 25.7% when combined to nasal sampling. It confirmed, however, that the anterior nares are the most colonized site with *S. aureus*. Our protocol included initially a rectal swabbing before surgery; however, since many patients denied this sample, the data regarding rectal specimens could not be taken into consideration. With a single nasal sample and in the absence of additional throat and rectal specimens, the frequency of carriage of *S. aureus* in our cohort was certainly underestimated, as illustrated by the figure of 20.2% of nasal carriers in this study compared to 37.1% in the one of Mertz et al [23] that combined nasal and throat samplings. Second, we obtained only one qualitative (and not quantitative) nasal sample from each patient. However, the most predictive factor for S. aureus infections is the persistent carriage pattern [24]. Getting up to seven nasal swab cultures to accurately segregate non-carriers from intermittent carriers, as suggested by Nouwen *et al*. [25], was not practically feasible for such a large effective. Indeed, most *S. aureus* screening programs require only one swabbing of the anterior nares [8, 13, 15]. Third, with an end-point at 1 year after the device implantation, we missed late infections (> 2 years) [26]. However, the latter infections are not systematically attributed to a per-operative contamination and are mostly caused by organisms other than *S. aureus* [4].

Fourth, and this is the major weakness of our study, many strains of *S. aureus* were not available for molecular typing because infection was often detected outside the surgical setting and the corresponding laboratory omitted to keep the strain.

Despite these criticisms, in our study, with the hypothesis of maximum bias, at least 16 of the 22 cases of *S. aureus* SSI were not related to *S. aureus* nasal carriage before surgery. The origin of *S. aureus* may be another site of carriage, such as throat [23], perineum or digestive tract [27, 28]. Indeed, throat or intestinal carriage without nasal carriage occurs relatively frequently [23, 27], which can provide a rationale to investigate this reservoir for endogenous origin of *S. aureus* infection. Orthopaedic SSI can also result from exogenous transmission, as it has been already shown, notably from the hospital environment and from health-care workers [29, 30]. It is noteworthy that, in our study, no cross transmission of *S. aureus* within centres was documented. Our results could explain why in general surgery [9] and in orthopaedic surgery [15], randomised controlled trials with mupirocin, eliminating solely the nasal carriage, were unable to reduce *S. aureus* SSI. Concerning the strategies to prevent SSI, in the more recent SHEA/IDSA practice recommendations, the routine screening for *S. aureus* carriage or attempts to decolonize surgical patients with an antistaphylococcal agent in the preoperative setting remains unsolved issues [31].

In conclusion**,** nasal carriage of *S. aureus* at the time of surgery, as investigated in this study, is a risk factor for *S. aureus* SSI in orthopaedic surgery. However, screening for *S. aureus* of the nares only, did not reliably predict *S. aureus* SSI. In most cases of *S. aureus* SSI, either an endogenous origin could not be demonstrated, or pre-operative nasal colonisation retrieved a strain different from the infecting pathogen. Further studies are necessary to elucidate the role of extra-nasal *S. aureus* carriage and the potential role of exogenous sources of contamination in elective orthopaedic surgery. As pointed out by our results, non *S. aureus* nasal carriers

must also be taken into account arguing for the importance of implementing robust standard prevention strategies for SSI.

STUDY GROUP MEMBERS

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Contributors

PB, CC and FL participated in the design of the study, in the collection, analysis, and interpretation of the clinical data, in the writing of the report and the decision to submit the paper for publication. FG and BP participated in the collection, analysis and interpretation of the microbiological data. JPP, JPF, JB, MHF, FF participated in various aspects of trial conduct and patient referral. All authors had full access to all of the data in this study and take complete responsibility for the integrity of the data and the accuracy of the data analysis.

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Legends of figures:

Figure 1: Frequency of surgical site infection according to the type of prosthesis implemented

Figure 2: *Sma*I PFGE profiles of strains of *S. aureus* isolated from four patients for whom the nasal isolate(s) and the surgical site isolate(s) were available. Strain of *S. aureus* NTCC 8322 was used as a size marker. * indicates the reference size marker, N : nasal strain, I : isolate from SSI, S : isolate from stools

Table I: Main characteristics of the 3908 patients included in the study according to the type of osteosynthetic surgery

Type of osteosynthetic surgery	Hip prosthesis	Knee prosthesis	Uni compartmental knee prosthesis	Others	Total
Number of patients (%)	2321 (59.4%)	1162 (29.7%)	292 (7.5%)	133 (3.4%)	3908 (100%)
Mean age (+/- SD)	67.7 (+/-10.8)	72.9 (+/-7.9)	72.7 (+/-7.2)	76.6 (+/-10.3)	69.9(+/- 10.0)
Male (%)	1121 (48.3%)	408 (35.1%)	91 (31.2%)	58 (43.0%)	1678 (43.0%)
Diabetes (%)	211 (9.0%)	159 (13.7%)	37 (12.7%)	13 (9.6%)	420 (10.7%)
Immunocompromised patients[a]	5 (0.2%)	5 (0.4%)	1 (0.3%)	0 (0%)	11 (0.3%)
Cigarette use	445 (19.2%)	152 (12.9%)	28 (9.6%)	17 (12.6%)	642 (16.4%)
Rheumatoid polyarthritis	50 (2.1%)	31 (2.7%)	2 (0.7%)	7 (5.2%)	90 (2.3%)
Antibiotic use one month prior	97 (4.2%)	49 (4.2%)	10 (3.4%)	8 (5.9%)	164 (4.2%)
Corticosteroids use	75 (3.2%)	35 (3.0%)	6 (2.1%)	6 (4.4%)	122 (3.1%)
Cancer	227 (9.8%)	130 (11.2%)	35 (12.0%)	16 (11.9%)	408 (10.4%)
Renal disease	8 (0.3%)	2 (0.2%)	0 (0%)	0 (0 %)	10 (0.3%)
Bacteriuria	50 (2.2%)	23 (2.0%)	4 (1.4%)	3 (2.2 %)	80 (2.1%)
Blood transfusion	182 (7.8%)	156 (13.4%)	1 (0.3%)	24 (17.8%)	363 (9.3%)
Notable hematoma	28 (1.2%)	16 (1.4%)	4 (1.4%)	0 (0%)	48 (1.2%)
Antimicrobial prophylaxis	2310 (99.5%)	1159 (99.7%)	292 (100%)	133 (100 %)	3895 (99.7%)
Cement use for prosthesis					
1- cement	58 (2.5%)	418 (36.0%)	25 (8.6%)	47 (33.8%)	548 (14.0%)
2 – partially cemented	248 (10.7%)	359 (30.9%)	7 (2.4%)	9 (6.8%)	623 (16.0%)
3 – no cement	2017 (86.8%)	384 (33.0%)	260 (89.0 %)	76 (57.1%)	2737 (70.0%)
Primo implementation	2254 (97.1%)	1134 (97.6%)	288 (98.6%)	13 (9.6%)	3689 (94.4%)
SSI frequency	2.2%	1.7%	0.7%	3.8%	2.0%
SSI frequency according to NNIS index					
0	1.5%	0.9%	1.0%	2.2%	1.3%
1	3.8%	3.3%	0%	5.3%	3.3%
2	4.9%	5.6%	0%	20.0%	6.6%
Frequency of SSI due to *S. aureus*	0.8%	0.2%	0%	2.3%	0.6%
Frequency of SSI due to *S. aureus* according to NNIS index 0	0.3%	0.1%	0%	2.3%	0.3%
1	2.0%	0.3%	0%	0%	1.3%
2	1.6%	0%	0%	20.0%	1.9%

[a] Immunocompromised patients: malignancy, transplantation, immunosuppressive therapy, HIV infection

Table II: Microbiological data

Bacterium	**Number**
Coagulase negative *Staphylococcus* spp.	36
Methicillin susceptible *S. aureus*	18
Methicillin resistant *S. aureus*	4
Enterobacteriacae	11
Enterococcus spp.	7
Streptococcus spp.	6
Propionibacterium acnes	3
Pseudomonas aeruginosa	2
Corynebacterium spp.	2
Pseudomonas paucimobilis	1
Clostridium perfringens	1
Fusobacterium spp.	1
Peptostreptococcus spp.	1
Acinetobacter spp.	1
Including polymicrobial infections	14

Table III: Detailed results of the 22 cases of SSI due to *S. aureus* and their relationship with nasal carriage.

Patient	Type of surgery	No. of days between surgery and SSI	Bacterial strain responsible for SSI[a]	Nasal sampling before surgery[a]	Other nasal samplings[a]	Classification of pairs of *S. aureus* isolated from the nose and the SSI
1	Hip prosthesis	15	MSSA (I4)	Positive (N3)		different (PFGE and AP)
2	Knee prosthesis	15	MSSA	Negative		
3	Hip prosthesis	26	MRSA	Positive	positive one month after SSI diagnosis	similar (AP)[b]
4	Hip prosthesis	22	MSSA	Negative		
5	Hip prosthesis	25	MSSA	Positive	positive 2 months after SSI diagnosis	different (AP)[c]
6	Hip prosthesis	25	MSSA	Negative	positive at the time of SSI diagnosis	
7	Hip prosthesis	30	MSSA	Negative		
8	Hip prosthesis	20	MRSA	Negative		
9	Partial hip prosthesis replacement	24	MSSA	Negative		
10	Hip prosthesis	19	MSSA	Negative		
11	Hip prosthesis	22	MSSA	Positive	positive 2 months after SSI diagnosis	similar (AP)[d]
12	Hip prosthesis	43	MSSA (I9)	Positive (N8)	nose sampling number 2 positive	similar (PFGE and AP)
13	Hip prosthesis	35	MSSA	Negative		
14	Hip prosthesis	20	MSSA	Negative		
15	Hip prosthesis	23	MSSA (I2)	Positive (N1)		similar (PFGE and AP)
16	Hip prosthesis	62	MSSA	Negative	2 negative samplings	
17	Knee prosthesis replacement	82	MRSA	Negative		
18	Knee prosthesis	41	MSSA	Positive	positive 13 days after SSI diagnosis	similar (AP)[e]
19	Hip prosthesis	42	MSSA	Positive	negative 2 months after SSI diagnosis	similar (AP)[f]
20	Hip prosthesis	73	MSSA	Negative		
21	Hip prosthesis	41	MSSA + *Klebsiella pneumoniae*	Negative		
22	Hip prosthesis	63	MRSA (I7) + *Pseudomonas aeruginosa*	Positive (MSSA) (N5)	positive MRSA (S6) in stools five days before SSI	Different (PFGE and AP)

SSI = surgical site infection; AP = antimicrobial profile; MSSA = Methicillin susceptible *S. aureus*; MRSA = Methicillin resistant *S. aureus* ; PFGE = Pulsed Field Gel Electrophoresis.

[a] Figures in brackets correspond to the numbering of *S. aureus* isolates in Fig. 2
[b] patient 3 harbored nasal and SSI MRSA strains that were both sensitive to all aminoglycosides but resistant to erythromycin and lincomycin.
[c] patient 5 harbored distinct *S. aureus* strains: the nasal strain was only resistant to penicillin G and intermediate to norfloxacin whereas the SSI strain was resistant to penicillin G and erythromycin and sensitive to norfloxacin.
[d] patient 11 harbored nasal and SSI MSSA strains that were both sensitive to all tested antibiotics except penicillin G.
[e] patient 18 harbored nasal and SSI MSSA strains both only resistant to penicillin G.
[f] patient 19 Nasal strain was only resistant to penicillin G although the SSI strain was resistant to penicillin G and intermediate to pristinamycin

Table IV: Univariate and multivariate analysis of risk factors for SSI overall and SSI due to *S. aureus*

Risks factors	**Univariate analysis**	**Multivariate analysis**	
For SSI overall	*P*	*P*	Exp (B) CI 95%
Centre	< 0.01	0.0379	Adjustment factor
Age	0.03		
BMI	< 0.001		
Tobacco use	0.003	0.0018	2.244 [1.352 - 3.726]
Diabetes	0.04		
Cancer	0.06		
Corticosteroids	0.1		
First implantation	0.07		
Duration of surgery	0.004		
Hematoma	0.002	0.0026	4.665 [1.714 - 12.695]
Nasal carriage of *S. aureus*	0.3		
NNIS	< 0.001	< 0.0001	3.073 [1.874 - 5.038]
ASA score > 2	< 0.01		
For SSI due to *S.aureus*			
Centre	NS	0.9978	Adjustment factor
ASA score > 2	< 0.01		
BMI	0.2		
Tobacco use	0.005	0.0024	3.907 [1.621 - 9.420]
Diabetes	0.025		
Cancer	0.02		
Duration of surgery	0.02		
Nasal carriage of *S. aureus*	0.02	0.0208	2.786 [1.169 - 6.640]
NNIS	< 0.001	0.0007	5.205 [2.013 - 13.455]

Authors' Response to Reviewers' Comments
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Dear Editor,

Please find enclosed a revised manuscript of our study entitled "Is nasal carriage of *Staphylococcus aureus* the main acquisition pathway for surgical-site infection in orthopaedic surgery?" Ms. No. EJCMID-D-09-00479 and the answers, point by point, to the queries of the two reviewers that are detailed below. The main changes done are highlighted in yellow in one of the copy of the new manuscript.

We hope that this new version will be found suitable for publication in your journal.
I look forward to hearing from you soon.
Sincerely yours,

Pr Berthelot
MD, PhD.

Reply to reviewers

Thank you for these interesting comments. Please find below our answers to the queries of the two reviewers.

Reviewer 1

It is unfortunate that they did not screen other sites such as the throat and perineum.

We agree with this comment. Initially, the protocol included a rectal swabbing but many patients were reluctant to this proposal. Due to a high rate of missing data during the initial step of the study, we decided to stop the sampling of this site. The following sentence was added in the discussion section of the new manuscript (page 11; lines 12 to 14): "Our protocol included initially a rectal swabbing before surgery; however, since many patients denied this sample, the data regarding rectal specimens could not be taken into consideration". Concerning the throat sampling, when the study was designed in 2002, sampling this site was not recommended since this specimen was not considered at this time to increase significantly the sensitivity of the recovery of *S. aureus* from the upper respiratory tract as shown later by Metz et al. in 2007 [ref 23] (this point is discussed page 11, lines 14 to 17).

They could point out that sole carriage at these sites is rare (perhaps 10%) and add this to their estimates of the possible size of the problem (20% larger!). Nasal carriage rather than carriage should be in the text.

We also agree with this comment, which is emphasised in the new discussion section (page 11; lines 14 to 17): "With a single nasal sample and in the absence of additional throat and rectal specimens, the frequency of carriage of *S. aureus* in our cohort was certainly underestimated, as illustrated by the figure of 20.2% of nasal carriers in this study compared to 37.1% in the one of Mertz et al [23] that combined nasal and throat samplings."

Comment 1 about CNS data:
1) Table 3 has overall data. They could compare the antibiograms of the individual isolates and see if there are very different strains. I doubt the organisms are available for molecular analysis (if so this could be "future work" for the discussion) but at least the antibiograms could be compared by ward and centre and see if there are any similarities and differences and informed guesses made as to the likelihood of cross infection e.g. quinolone resistance is usually mutational
2) All the other infected organism data. Again what about antibiograms and possible cross infection?

The goal of the study was to address the potential link between *S. aureus* nasal carriage and occurrence of *S. aureus* SSI, and not to study CNS or other bacterial cross infections. Strains other than *S. aureus* were not kept for further analysis. By contrast, the potentiality of cross infections with *S. aureus* was investigated and presented with some details in the revised manuscript (see below).

3) S aureus data.
We need to see their rule set for deciding why the extra 5 strains were different or similar. This is a difficult area and must be explained.

The way used to compare antibiotypes is reported in the method section (page 6, lines 12 to 16). As required by the referee, additional information regarding the comparison of antibiotic profiles has been added in the footnotes of Table III.

They should compare the 13 SA from non carriers (and indeed the carriers) to see if there are possible issues of cross infection in terms of time person and place of the affected patients. Are these strains similar by PFGE? Are they similar to any of the carriers?

From the 13 *S. aureus* SSI strains isolated from non nasal carriers, 8 were available for comparison by PFGE: all these strains exhibited independent profiles, also different from those of strains isolated from the nose of colonised patients. The 5 remaining strains were isolated from non nasal carriers hospitalized in 5 different centres, excluding cross transmission. These data were added in the result section page 9 lines 10 to 13.

Table IV only has death related risk analysis not as stated on page 10 lines 23-24 for SSI and SA nasal carriage.

Because of the low number of SSI infections, the multivariate analysis was run with a maximum of 3 covariates selected among the most significant in the univariate analyses. This sentence was added in the method section (page 8 lines 8 to 10). We also modified the sentence presenting Table IV (page 10 lines 14 to 15).

4) There are also the therapeutic issues raised by the AST results for all categories of infections above. These are perhaps a little peripheral but I think it would add to the paper or perhaps be another one if there are clinical outcome data?

Data regarding therapeutic issues are not presented in this paper. Another clinical paper focusing on these results is in preparation

Other points
How were nasal swabs taken: were the swabs moistened?

The precision regarding moistened transport medium was added in the method section (page 5, line 1).

Was the PFGE protocol validated to be able to distinguish strains and how?

The criteria for PFGE interpretation are described page 6 lines 10 to 12 "For a same patient, according to Tenover's criteria [18], two *S. aureus* strains were classified as epidemiologically distinct if a difference of more than three bands was ascertained between the PFGE profiles performed in a same run".

Reviewer 2

First this was a multicenter study and in these kind of studies cluster effects may occur. In the multivariate analysis, adjustment for cluster effects was not included. This would strengthen the conclusions.

As pointed by the reviewer it is important to look for cluster effect. This was investigated by adding a "centre" variable in the different statistical analyses. This variable did not remain in the final model used for multivariate analysis. But as required by the referee, we performed another multivariate analysis using windows SAS® 9.1 software to adjust for cluster effect. This was added in the method section (page 7, line 25). We replaced the results of the previous multivariate analysis performed using SPSS software by the results of this multivariate analysis adjusted for cluster effect (Table IV).

Second, the authors conclude that the majority of the *S. aureus* strains are of exogenous origin. They put it quite strong in the conclusions that 'at least 16 were not endogenous'. In my opinion this is not correct. The nasal cultures were negative but this may have been a false-negative result, e.g. due to the sampling technique. As the carriage rate was only 20% it is clear that the culture technique was not very sensitive (normally 30% of the population is nasal carrier). Therefore, I suggest to modify this conclusion.
In the majority of cases an endogenous origin could not be demonstrated would be more correct.

We agree with this comment. The discussion section was amended accordingly (see answer to reviewer 1 and additional sentence in page 11; lines 14 to 17)).
As suggested by the referee, we also modified the conclusion; the sentence "In most cases of *S. aureus* SSI, pre-operative nasal colonization was not documented or retrieved a different strain from the infecting pathogen" was replaced by the following one in the new manuscript (page 12, lines 20 to 22 and in the abstract): "In most cases of *S. aureus* SSI, either an endogenous origin could not be demonstrated, or pre-operative nasal colonisation retrieved a strain different from the one recovered from the surgical site"

Also, it would be important to provide information on the typing of the strains from non-carriers as well. I wonder whether there were many similar strains within centers suggesting a common source?

This point was also addressed by reviewer 1. No cross transmission of *S. aureus* was documented in this cohort. This important point was added in the result and discussion section, page 9 lines 10 to 13. The following sentence was added page 12 line 11 to 12 “It is noteworthy that, in our study, no cross transmission of *S. aureus* within centres was documented”.