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Unexpected categories at risk of *S. aureus* nasal carriage among hospital workers

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ABSTRACT

Objectives. Thirty percent of the general population are *Staphylococcus aureus* nasal carriers. It has been shown that this increases with repeated contact with patients, but it is not known whether all categories of healthcare workers are at equal risk of carriage. We aimed to explore *S. aureus* nasal carriage among healthcare professionals.

Methods. Prospective study conducted in two French university hospitals in 2014 and 2016. Volunteers were screened for *S. aureus* nasal carriage. Profession and hygiene habits were collected. Based on the results of this initial study, a second study focused on semi-skilled workers and biomedical equipment technicians (BETs) only; participants were given education on the basic rules of hygiene, then re-screened three months later.

Results. In the initial study, 38.8% of the 436 participants were detected as nasal carriers. There was a significant difference in nasal carriage according to professional category ($p<0.0001$); the lowest was found among administrative agents (17.3%), followed by healthcare providers (37.4%), laboratory technicians (37.6%). The greatest proportion was found among semi-skilled workers and BETs (52.9%). *Spa*-typing ruled out the hypothesis of a single clone dissemination among colleagues. After the three-month hygiene awareness campaign, all re-screened individuals remained positive, and with their respective initial strain.

Conclusions. To the best of our knowledge we report here for the first time that semi-skilled workers and BETs are specifically more at risk of *S. aureus* nasal colonisation. This striking finding urges hospital hygiene departments to evaluate this specific professional category and implement strategies to improve hygiene awareness.

Keywords: *Staphylococcus aureus*; nasal carriage; healthcare workers

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is known as a major human pathogen causing a wide range of nosocomial and community-acquired infections. Other than its pathogenicity, *S. aureus* is also a human commensal that is retrieved from many different body sites such as the nose, throat, digestive tractus, perineum, and skin surface (Acton et al., 2009; Bourgeois-Nicolaos et al., 2010; Claassen-Weitz et al., 2016; Wertheim et al., 2005). Notably, the nares represent the main reservoir of *S. aureus* carriage; around 30% of the general population being colonised (Kluytmans et al., 1997). It has been shown that some individuals host *S. aureus* on a regular basis (known as persistent carriers), while other carriers have only a transient colonisation (intermittent carriers) (Wertheim et al., 2005). It is well known that *S. aureus* nasal carriage is associated with an increased risk of developing subsequent *S. aureus* infections (Eiff et al., 2001; Wertheim et al., 2005), in particular for persistent carriers (Verhoeven et al., 2014), in both community-acquired and healthcare associated infections (Kluytmans et al., 1997; Muñoz et al., 2008).

Nasal colonisation rate relies on both host (immune response, nasal microbiota, antimicrobial peptides release) and bacterial (adhesins, ability to survive in stress or deprived conditions) determinants (Johannessen et al., 2012; Peacock et al., 2001). Some risk factors for nasal colonisation have been extensively described and include promiscuous living conditions (military conditions, fire station, *etc*…), older age, male sex, or patients undergoing haemodialysis (Cole et al., 2001; Heijer et al., 2013; Herwaldt et al., 2004; Yu et al., 1986). While repeated exposure to *S. aureus* due to frequent contact with patients or microbiological substances may increase carriage risk (Okamo et al., 2016; van Vugt et al., 2015), it has been described that the compliance with basic hygiene recommendations, including alcohol-based hand rub solution (AHS), reduces the risk of nasal colonisation (Gould et al., 2018; Saadatian-

Elahi et al., 2013). However, few studies have explored *S. aureus* nasal colonisation and inherent risk factors among the various categories of hospital staff. A recent paper has reported that medical technologists (*i.e*. those working in operating rooms, chemotherapy, and imaging) had a higher nasal carriage rate than other healthcare workers (HCW) in the same hospital in Argentina (Boncompain et al., 2017). Also, van Vugt *et al.* found that *S. aureus* nasal carriage rate was significantly greater among surgeons compared to non-hospitalised individuals (van Vugt et al., 2015). These elements suggest that the hospital setting may be a favouring condition for *S. aureus* nasal carriage, but with disparities between HCW categories, and *S. aureus* transmission by colonised HCW. The aim of the present study was to explore *S. aureus* nasal carriage among different HCW categories in two independent tertiary hospitals in France.

METHODS

Written informed consent was obtained from all participants. The studies were conducted in accordance with the declaration of Helsinki, French national regulations, and approved by the local ethics committee that considered the study as non-interventional. Relevant approval (reference RPD09891439) regarding access to patient-identifiable information was granted by the French data protection agency (*Commission Nationale Informatique et Liberté*, CNIL).

Information posters advertised the study throughout two French university hospitals (Lyon and Grenoble, in April 2014 and June 2016, respectively), inviting all individuals among the personnel to participate. Those who volunteered underwent nasal swabbing on one of two consecutive days. Double plastic swabs were used; each nostril was sampled by both swabs (Copan, Brescia, Italy). *S. aureus* carriage was screened by real-time PCR (GenXpert® SA Nasal Complete, Cepheid, Maurens-Scopont, France) with the first swab. For positive

samples, the second swab was subsequently cultured on ChromAgar® (I2A, Montpellier, France) and blood agar plate (bioMérieux, Marcy-l'étoile, France; 35°C, 48h). Identification of suspect colonies was confirmed by either both catalase and latex agglutination tests (Slidex, bioMérieux, Marcy-l'étoile, France) or MALDI-TOF. *S. aureus* strains were stored at -20°C. An anonymous questionnaire collecting demographic data, profession, risk factors like haemodialysis, immunosuppressive treatment, diabetes mellitus, and type of contact with patients was filled out by each participant. Profession categories were grouped as follows: (i) HCWs without direct contact with patients and without exposure to the hospital or laboratory environment (*i.e.* director of human resources, clinical research associate, lawyer, *etc*…) hereafter termed "administrative agents", (ii) HCWs with direct contact with patients and exposed to the hospital environment (*i.e.* nurses, physicians, physical therapists, dieticians, radio technicians, psychologists, stretcher bearers, *etc*…) hereafter termed "healthcare providers", (iii) HCWs without direct contact with patients and exposed to the laboratory environment hereafter termed "lab technicians" and (iv) HCWs without direct contact with patients and exposed to the hospital environment (*i.e.* semi-skilled workers such as carpenters, electricians, plumbers, and biomedical equipment technicians (BETs) in charge of the repair and maintenance of medical instruments) hereafter termed "semi-skilled workers and BETs".

A second study focusing on semi-skilled workers and BETs only, was conducted in 2015 in Lyon, for which volunteers underwent nasal swabbing based on the same design as the initial study. To maximise the sensitivity of the screening, cultures were systematically performed along with molecular testing. A worker was considered as carrier when at least one of the two techniques was positive. The anonymous questionnaire was enriched with an item on hand hygiene habits. Following the initial nasal swabbing, participants were given weekly educational sessions on basic recommendations of hand hygiene by the hospital hygiene team and the occupational health department and were supplied with individual bottles of AHS

(replaced on demand) for a 3-month period. A final nasal screening was performed after three months. *S. aureus* strains isolated from positive samples were characterised by *spa*-typing according to the Ridom Staph Type standard protocol (http://www.ridom.com).

Categorical variables were reported as number and percentage, and continuous variables are reported as median and interquartile range [IQR]. Participant characteristics were compared using Chi-squared or Fisher's exact tests for categorical variables. Quantitative variables were compared using Mann-Whitney tests. All statistical tests were two-tailed and a P value ≤ 0.05 was considered statistically significant. Statistics was performed with Stata, version 12 (Stata Corp, Texas, USA). Because gender influences *S. aureus* carriage rate and the sex ratios differed between professional groups, we determined whether between-group differences could result from sex ratio differences. The associations between *S. aureus* carriage and professional group were analyzed in a mixed-effect logistic model including patient sex and the hospital (Grenoble or Lyon) as random effects. Computations were performed using packages *lme4* and *lmerTest* for the R environment version 3.6.0.

RESULTS

A total of 309 and 157 HCWs participated in the study in Lyon (2014) and Grenoble (2016). Respectively, 7 and 16 participants in Lyon and Grenoble were excluded due to invalid molecular tests; a further 7 participants in Lyon were excluded due to missing questionnaires. A total of 436 individuals were thus included in the study, 295 individuals in Lyon and 141 in Grenoble. Two-thirds (67.7%) of the total population (68.4% in Lyon, 66.4% in Grenoble) were women. The median age was 43 years (IQR [31-53]) and this was significantly higher for participants in Lyon (45 years, IQR [33-54]) than Grenoble (39 years, IQR [27-50]; p =

0.0012)(Table 1). Participants were distributed as follows: 41/578 and 11/482 administrative agents, 94/4100 and 53/4155 healthcare providers, 109/140 and 24/277 lab technicians, and 51/380 and 53/242 semi-skilled workers and BETs in Lyon and Grenoble, respectively.

Overall, 38.8% (n=169) of the participants were detected as *S. aureus* nasal carriers, with a significantly higher proportion in Grenoble (46.8%, n=66) than in Lyon (34.9%, n=103; p=0.02, Table 1). Only 2 strains were detected as methicillin-resistant *S. aureus* (MRSA) in Lyon (0.7%), and none in Grenoble.

There was a significant difference in the proportion of nasal carriage according to professional category (p<0.0001); the lowest proportion was found among administrative agents (17.3%), followed by healthcare providers (37.4%), and laboratory technicians (37.6%). Unexpectedly, the greatest proportion was found among semi-skilled workers and BETs (52.9%; Table 1). These results were confirmed when controlling for sex and sampling location. Indeed, compared to the healthcare providers, the frequency of *S. aureus* carriage was similar in laboratory technicians (adjusted OR=0.95, 95% CI 0.56 to 1.61 p=0.86), lower in administrative agents (adjusted OR=0.35, 95% CI 0.15 to 0.91, p=0.015), higher in semi-skilled workers and BETs (adjusted OR=1.77, 95% CI 1.02 to 3.06, p=0.042).

To explore further this observation, the second study focused on this specific HCWs category in Lyon in 2015. Among the 44 semi-skilled workers and BETs who participated, 23 (52.4%) were *S. aureus* nasal carriers (none MRSA) at the initial swabbing (M0), confirming the results of the initial study (Figure 1); 20 were detected by both PCR and culture, and 3 by culture only (2 invalid and 1 discordant negative molecular tests).

At M0, the 23 isolates belonged to 19 different *spa*-types (Figure 2). No predominant *spa*-type was observed; a maximum of two individuals harbouring the same *spa*-type (t2, t148, t342, and t1451)(Table 2). After 3 months, 22 of the 44 participants accepted to be re-

screened; 14 of which were initially known as carriers and 8 as non-carriers. All the 14 carriers were still positive for nasal *S. aureus* after 3 months, 6 of the 8 non-carriers remained negative, and 2 acquired *S. aureus* over the 3-month period (Figure 1). Characterisation of the 16 isolated strains by *spa*-typing found 12 different spa-types (2 failures); the 14 participants who were still detected positive harboured their respective initial strain, based on *spa*-type (Table 2).

All the 44 semi-skilled workers and BETs enrolled in this second study declared having no direct contact with patients or microorganisms. All of them also affirmed "never" or "rarely" wearing gloves and using AHS when on duty. After 3 months, 90.9% (n=20) of the participants who were re-screened declared now using AHS "often" or "always" after each intervention.

DISCUSSION

Although *S. aureus* nasal carriage is extensively explored, few studies have investigated *S. aureus* nasal colonisation and inherent risk factors among HCWs. The present study found an overall carriage rate of 38.8% in two French university-hospital personnel, which is above usually stated rates in the general population, which is around 30% (Kluytmans et al., 1997; Lepelletier and Lucet, 2013).

Strikingly, when stratifying by professional categories, we found that semi-skilled workers and BETs had a significantly higher carriage rate, with about half of the group being colonised. To the best of our knowledge, this is the very first time that such a professional category has been investigated. Interestingly, similar results were obtained in the second study, and in both independent hospitals, strengthening the validity of this finding. Data were controlled for sex and hospital site, ruling out such possible biases. Other risk factors like

haemodialyssis, immunosuppressive treatment or diabetes mellitus were exceptional among participants, ruling out a confounding factor.

Moreover, the hypothesis of a single clone dissemination among professionals was ruled out since a wide variety of *spa*-types were observed in this group. One may hypothesise that such hospital professionals, while exposed to a microbiologically contaminated environment on a daily basis, do not comply with hygiene recommendations as strictly as medical and paramedical staff. Strikingly, all the semi-skilled workers and BETs declared in the second study no direct contact with patients or microbiological agents, while in fact working within the patient care environment when intervening in patient rooms or medical wards for technical reasons. Moreover, at the beginning of the second study, all declared "never" or "rarely" wearing gloves or using AHS when on duty. This highlights the under-recognition of the microbiological risk associated with their conditions of work. The combination of both exposure to a contaminated environment and lack of knowledge and compliance with hand hygiene rules may be determinants for such a high carriage rate among these professionals. This is supported by the finding that healthcare providers, who are in direct contact with patients but made aware of importance of hand hygiene, displayed a lower carriage rate. In addition, administrative agents who work in a separate dedicated building, and thus have no direct contact with patients and no exposure to the hospital environment, showed the lowest carriage rate.

The impact of the 3-month educational course on hygiene was evaluated by both the self-reported hygiene habits and their rate of nasal carriage. Almost all of the participants declared increased hygiene compliance over the 3-month period, with a virtually systematic use of AHS after a technical intervention. Nevertheless, there was no decrease of carriage rate over the study period since all the carriers who underwent nasal swabbing at both inclusion time and 3 months later remained positive. Since persistent carriers represent only 20-25% of the

carrier population (Verhoeven et al., 2012), the lack of impact of the 3-month educational course on hygiene could not be attributed to such a hypothesis. However, as the change in hygiene habits over the study period was only based on participant declaration, the accuracy of such declarations and the effective progress of compliance to basic hygiene recommendations could be questioned. More generally, the results of the present study lead to the question of the risk of *S. aureus* transmission in the hospital setting posed by such colonised workers, but also the determinants of *S. aureus* nasal carriage, which could include socio-economic status and the level of education that may be of interest to investigate in future studies.

Regarding the limitations of the study, it would be interesting to perform similar studies at a greater-scale. Also, we are aware that some people would more gladly participate than others, which could induce a bias, even though we considered that such volunteers would not be associated with a significantly different carriage rate.

To the best of our knowledge we report here for the first time that semi-skilled workers and BETs are specifically more at risk of *S. aureus* nasal colonisation. This striking finding urges hospital hygiene departments to evaluate this specific professional category and implement strategies to improve hand hygiene awareness.

DECLARATION OF INTEREST

None

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FIGURE CAPTIONS

Figure 1. Flowchart of semi-skilled workers and biomedical equipment technicians participating in the second study (Lyon, 2015). Volunteers among semi-skilled workers and BETs underwent nasal swabbing and were then given weekly educational sessions on basic recommendations of hand hygiene for a 3-month period. A final nasal screening was performed after three months.

Figure 2. Distribution of the 23 *S. aureus* strains isolated from of semi-skilled workers and biomedical equipment technicians participating in the second study (Lyon, 2015) based on spa-types. Each circle represents a carrier participant. Among the 23 *S. aureus* carriers, 19 different *spa*-types were detected, 10 of which were singletons.

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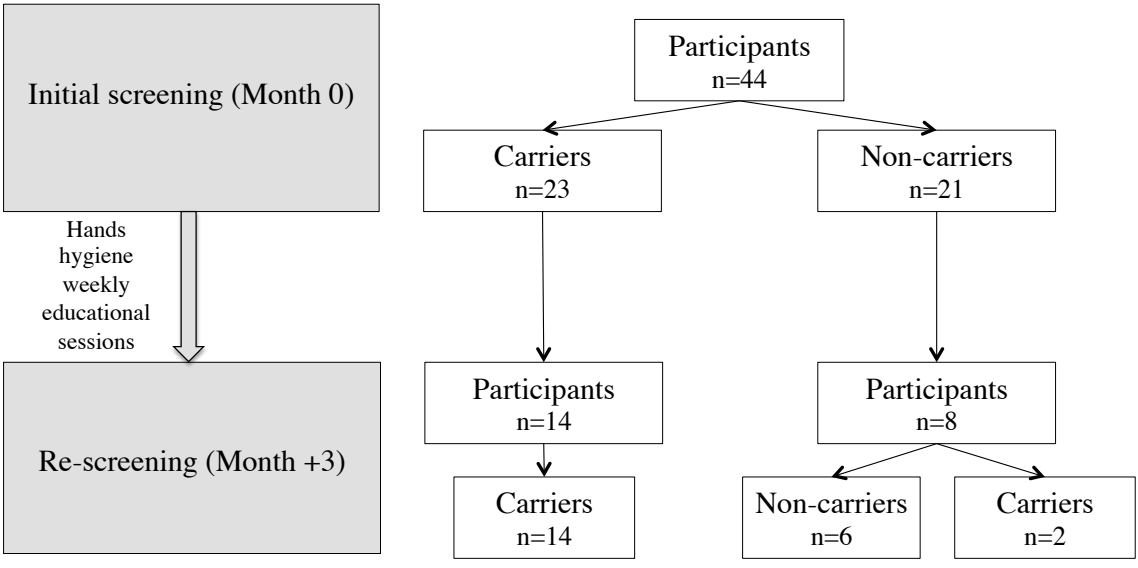
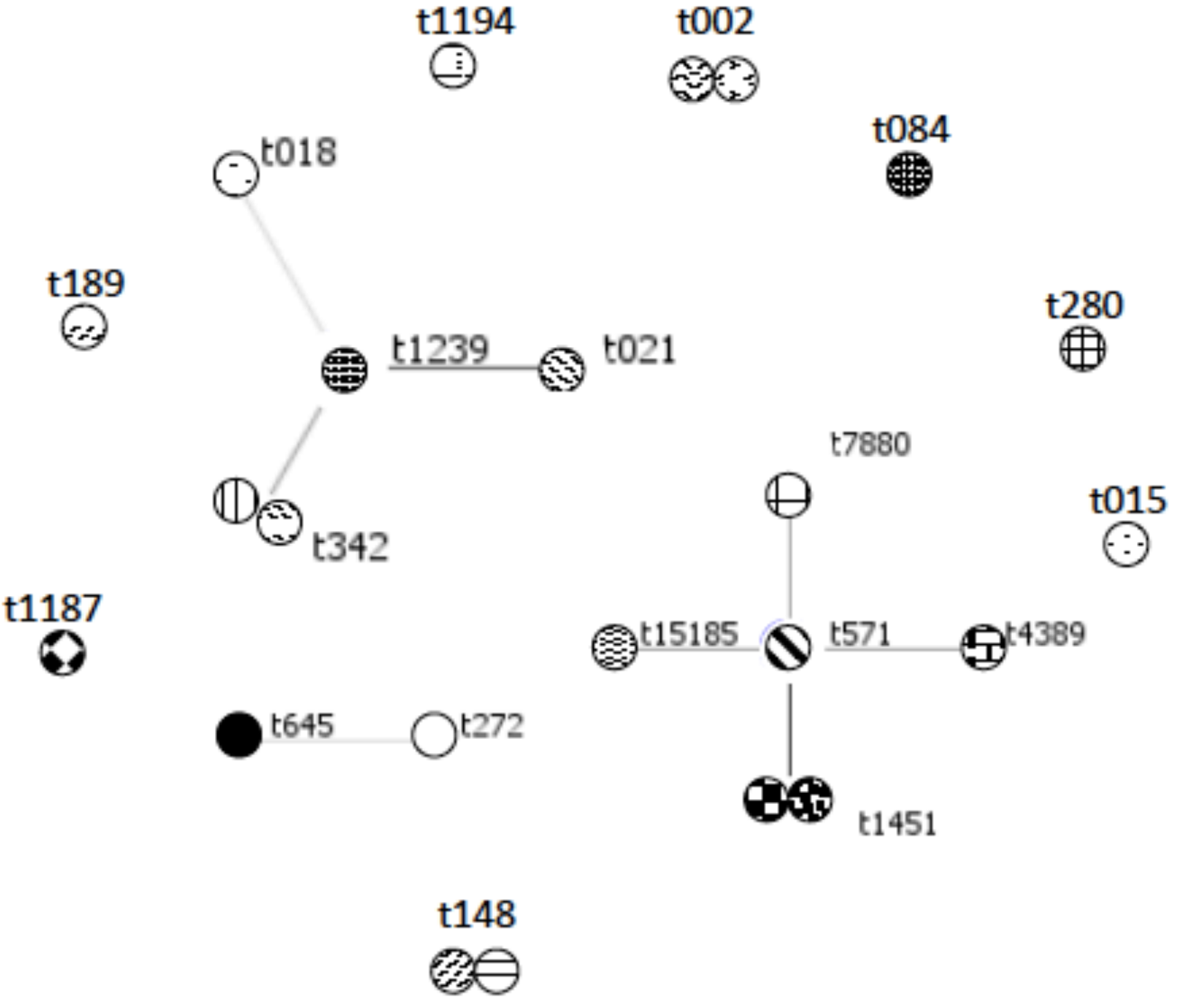

Initial screening (Month 0)
Hands hygiene weekly educational sessions
Re-screening (Month +3)
Participants n=44
Carriers n=23
Non-carriers n=21
Participants n=14
Participants n=8
Carriers n=14
Non-carriers n=6
Carriers n=2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t272
t1451
t148

Table 1. Characteristics of participants in the first study (Lyon 2014 and Grenoble 2016)

	Total population n=436	**Hospital**		*P* value
		Lyon n=295	**Grenoble** n=141	
Female sex, n (%)	275 (67.7)	186 (68.4)[(a)]	89 (66.4)[(b)]	NS
Median age, years [IQR]	43 [31-53]	45 [33-54][(c)]	39 [27-50][(d)]	0.0012
Nasal carriers, n (%)				
Administrative agents[(e)]	9 (17.3)***	7 (17.1)	2 (18.2)	NS
Healthcare providers[(f)]	55 (37.4)***	34 (36.2)	21 (39.6)	NS
Lab technicians	50 (37.6)***	39 (35.8)	11 (45.8)	NS
Semi-skilled workers[(g)] and BETs	55 (52.9)***	23 (45.1)	32 (60.4)	NS
Total	169 (38.8)	103 (34.9)	66 (46.8)	0.02

Abbreviations: IQR : interquartile range; NS : non significant; BETs : biomedical equipment technicians.
[(a)] 23 missing data
[(b)] 6 missing data
[(c)] 26 missing data
[(d)] 6 missing data
[(e)] director of human resources, clinical research associate, jurist, *etc*…
[(f)] nurses, physicians, physical therapists, dieticians, radio technicians, psychologists, stretcher bearers, *etc*…
[(g)] carpenters, electricians, plumbers and biomedical equipment technicians
*** $p<0.0001$. The rate of nasal carriage in the semi-skilled workers and BETs group was significantly higher than in each other group.

Table 2. Characterisation by *spa*-typing of strains carried by semi-skilled workers and biomedical equipment technicians in the second study (Lyon, 2015)

Participant ID number	*Spa*-type at Month 0	*Spa*-type at Month +3
1 and 6	148	NRS
2	280	NRS
3	272	NRS
4	15185	15185
5	15	15
7	21	NRS
8	571	571
9	189	NRS
10	18	failure
11	645	NRS
12	15187	15187
13 and 22	1451	1451
14	342	342
15	1239	1239
16 and 21	2	2 and NRS
17	7880	7880
18	1194	1194
19	342	342
20	4389	NRS
23	84	84
24	N/A	148
25	N/A	failure

Abbreviations: N/A: Not Applicable (non-carrier participant); NRS: participant not re-screened

Failure: no *spa*-type assignable