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The turnover of strains in intermittent and persistent nasal carriers of *Staphylococcus aureus*

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Summary *Objectives:* We aimed to examine the dynamics of *Staphylococcus aureus* nasal carriage in healthy adults.
Method: Selected *S. aureus* strains isolated from weekly nasal swabs obtained from 122 healthy young adults over a 13 week period were *spa* typed.
Results: The median duration of intermittent carriage was 4 weeks (IQR 2—6) and the median interval between episodes of carriage of different *spa* types was 3.5 weeks (IQR 2.25—4). 6/19 (32%) Persistent carriers were colonised with more than one *spa* type during the study, and in two persistent carriers a brief period of mixed colonisation with two *spa* types was observed. Even when the carriage strain changed, it was very rare for persistent carriers to have a period during which they were culture-negative (only 6/188 (3%) swabs submitted by persistent carriers failed to culture *S. aureus*).
Conclusions: Our results imply that at least every eight weeks a healthy young adult is exposed to *S. aureus* sufficient to cause a new episode of carriage among intermittent carriers. Persistent carriers are almost always colonised with *S. aureus* and over the course of a year there will be at least one replacement of the dominant strain.

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Introduction

Longitudinal studies of *Staphylococcus aureus* carriage by humans conducted before the era of molecular strain typing indicated three different groups: persistent *S. aureus* nasal carriers (approximately 20% of the population), intermittent *S. aureus* carriers (approximately 30% of the population), and non-carriers of *S. aureus*.[1] Although asymptomatic carriage of *S. aureus* occurs at various other sites, including the oropharynx[2–4]; none of these other sites are as strongly associated with the risk of staphylococcal disease as the anterior nares. Persistent nasal carriers are at increased risk of disease[5–8] and treatment to eradicate *S. aureus* nasal carriage provides a short-term reduction in the risk of disease.[9,10] Although efforts to reduce disease by eliminating nasal carriage should, arguably, focus on persistent carriers, there is no consensus regarding the definition of a persistent *S. aureus* nasal carrier.

Most studies have defined a persistent nasal *S. aureus* carrier as someone whose carrier index (the number of nasal swabs positive for *S. aureus*/total number of nasal swabs obtained) is greater than 0.8[6,11–13]; however, higher[14] or lower[2] carriage indices have been used. Two studies have found that the density of *S. aureus* cultured from nasal swabs is higher in persistent carriers than in intermittent carriers,[11,14] suggesting that persistent carriers might be identified by culturing two nasal swabs collected a few days apart.

Recent studies that performed molecular typing of *S. aureus* strains isolated from intermittent and persistent nasal carriers have added to our understanding of persistent nasal carriage. A study of Malaysian medical students identified twelve students (12/162, 7%) who had *S. aureus* isolated from nose or throat swabs on two occasions a month apart.[15] However, in each of these twelve students, the initial and subsequent isolates had different *spa* types. Another study of young children found that 68/268 (25%) had *S. aureus* isolated from each of two or three nasopharyngeal aspirates collected during autumn, winter and spring.[16] However, while in 40/68 (59%) of these persistently infected children the initial and subsequent isolates had identical or very similar *spa* types, in 28/68 (41%) the initial and subsequent isolates had different *spa* types. Other studies of *S. aureus* carriage have shown that some persistent nasal *S. aureus* carriers are colonised with the same genotype over prolonged periods[13,16,17]; however, other persistent carriers are colonised with a mix of genotypes or change genotypes over intervals of a few months.[17–19] These findings indicate that nasal *S. aureus* carriage is more dynamic than previously thought, and that strain replacement commonly occurs amongst people who would be defined as persistent nasal carriers based on their high culture index.

In contrast to previous studies, which commonly have collected sequential nasal swabs at intervals of a month or more, we sought to examine the dynamics of *S. aureus* nasal carriage in isolates obtained at weekly intervals from healthy volunteers. We aimed to measure the duration of episodes of carriage, and the duration of intervals between episodes of carriage, in intermittent carriers; and to examine the frequency of strain replacement in persistent carriers.

Healthcare workers, including medical students, have commonly been the subjects of studies of *S. aureus* carriage, due to their increased exposure in healthcare settings.[15,20–22] In the current study we examined a cohort of pre-clinical medical students as representative of healthy adults who frequently interact with each other, but who don't have extensive healthcare exposures.

Materials and methods

Participants, and nasal swabs

Ethical approval for the study was provided by the Northern Y NZ Ministry of Health Ethics Committee.

In August 2011, a class of 206 third-year medical students were invited to take part in the study, before they began any clinical attachments. The study was introduced to the class during a lecture about *S. aureus* disease. Students who agreed to take part provided written informed consent and were asked to complete a short paper questionnaire that collected information about demographics, past infections caused by *S. aureus*, healthcare and antimicrobial exposure, household details, and concurrent illness. Finally, the lecturer demonstrated nasal swab collection and students were instructed to self-collect a swab of both nostrils, prior to placing the swab into the transport tube containing Amie's gel media (Copan, CA, USA).[23,24] Each Friday at the end of a lecture, participating students were asked to provide a further self-collected swab. Students were also reminded weekly to inform the investigators if they became unwell, visited a healthcare professional or were treated with antimicrobial agents.

Weekly nasal swabs were collected over 13 weeks of the semester, except during a two-week mid semester break. Students were included in the study if six or more swabs were provided.

Definitions

A persistent *S. aureus* carrier was defined as someone who was culture-positive in at least 80% of swabs.[7,11,12] A non-carrier was defined as someone who never grew *S. aureus*. An intermittent carrier was defined as someone whose swabs cultured *S. aureus* on $<$80% of occasions.

The interval between new episodes of carriage in intermittent carriers was determined by counting the number of weeks in which culture-negative swabs were submitted by students following a prior period of carriage, and before a subsequent period of carriage. If the *spa* types isolated during the prior and subsequent periods of carriage were identical, then two or more sequential negative weekly swabs were required to document clearance. If the *spa* types isolated during the prior and subsequent periods of carriage were not identical, then one culture-negative swab was sufficient to document clearance. The duration of intermittent *S. aureus* carriage was determined by counting the number of weeks in which culture-positive swabs were submitted by students who had one or more culture-negative swabs both before and after the period of carriage. In some students the estimate of the duration of carriage incorporated the mid-term break and weeks

when no swabs were submitted. Carrier index was defined as the proportion of each carrier's swabs that cultured *S. aureus*.

Culture and *spa* typing of *S. aureus*

Each swab was inoculated onto mannitol-salt agar (Fort Richard, Auckland, NZ) within 6 h after collection. Plates were inspected after 48 h of incubation in aerobic conditions at 37 °C, and the identity of colonies displaying characteristics of *S. aureus* was confirmed using a BBL Staphyloslide latex agglutination test (BD, NJ, USA). DNA was extracted from a single *S. aureus* colony using a DNeasy kit (QIAGEN, Hilden, Germany). For all weeks, apart from week one, several streaks of bacterial growth (*S. aureus* and non-*S. aureus* colonies) were collected from the initial inoculation plate, using a sterile wire loop, and stored in 33% vv Glycerol in tryptic soy broth at −80 °C. These stored cultures were subsequently re-cultured on mannitol-salt agar and *S. aureus* colonies were randomly selected for *spa* typing using established methods.[25] Information regarding the molecular epidemiology of each *spa* type was obtained from: http://www.ridom.de/spa-server, accessed March 22nd 2015.

Selection of *S. aureus* isolates for *spa* typing

In all subjects with either intermittent or persistent carriage, single *S. aureus* isolates from the first and last positive swabs were *spa* typed. In those subjects with persistent carriage, who had identical *spa* types isolated from the initial and final swabs, we also *spa* typed twelve *S. aureus* isolates from either week five or week six, to determine whether carriage was exclusively with the same *spa* type throughout the duration of carriage. In those subjects with persistent carriage, who had different *spa* types isolated from the initial and final swabs, we also *spa* typed a single *S. aureus* isolate from every *S. aureus* positive sample. To determine whether the transition from carriage of one *spa* type to carriage of another *spa* type included a period of co-carriage of more than one *spa* type, we also *spa* typed twelve *S. aureus* isolates from both the week before and the week after the transition in *spa* types.

Analysis

Medians and interquartile ranges (IQR) have been calculated for non-continuous variables. The chi-square (categorical variables) and Kruskal–Wallis one way analysis of variance (age) tests were used to compare host characteristics between different carriage groups.

Results

146/206 (71%) Students agreed to take part in the study; however, six students provided no swabs and 18 provided less than six swabs and were excluded from further analysis.

The remaining 122 students provided 1181 nasal swabs over the course of the study; 12 swabs were not labelled and were discarded. The median number of weekly swabs provided by the 122 students included in the study was nine (IQR 8–11). During the study, *S. aureus* was isolated from 298/1181 (25%) nose swabs from 69/122 (57%) students. Using our definitions, 53/122 (43%) students were non-carriers of *S. aureus*, 50/122 (41%) students were intermittent carriers of *S. aureus* and 19/122 (16%) students were persistent carriers of *S. aureus*.

The median age (21 years, IQR 20–22 years) and sex (68/122, 56% were female) of the students did not differ between carriage status groups (Supplementary Table). Likewise, the number of students, who reported a history of exposure to community or hospital healthcare in the previous 12 months, did not differ between carriage status groups. In total 6/122 (5%) of the students reported a history of *S. aureus* illness. Persistent carriers were more likely to have a history of *S. aureus* illness (5/19, 26%) than intermittent carriers (3/50, 6%) or non-carriers (2/53, 4%) (chi-square $P < 0.05$). Household size, household crowding, household cigarette smoking; personal history of asthma, eczema, hayfever or food allergy; recent use of antibiotics, nasal sprays or inhalers; recent alcohol intake; and number of hours of weekly exercise did not differ between carriage status groups.

Dynamics of *S. aureus* nasal carriage

In total, 43 different *spa* types were isolated from 69 *S. aureus* carriers. The most common *spa* types identified were t189 (12 episodes of carriage), t002 (9 episodes), t127 (7 episodes), and t019, t091, t224 (3 episodes each). The corresponding MLST designations of these *spa* types all differ and these *spa* types are globally distributed.

The median duration between entry into the study and the first episode of carriage in the 50 intermittent carriers was 2.5 weeks (IQR 1–4 weeks); thus, the majority of the intermittent carriers were identified after four weeks. The average carrier index (proportion of swabs that cultured *S. aureus*) among intermittent carriers was 0.3 (range 0.09–0.7). There was a clear delineation between the carrier indices of the intermittent carriers and the persistent carriers (Supplementary Figure). The 50 intermittent carriers had 82 episodes of intermittent carriage. Twenty-two students had a single episode of carriage, 24 had two episodes of carriage and four had three episodes of carriage. There were 32 intervals between episodes of carriage: six intervals of one or more weeks (median 3.5 weeks, IQR 2.25–4 weeks) between episodes of carriage of different *spa* types; and 26 intervals of two or more weeks (median 5 weeks, IQR 4–6.75 weeks) between episodes of carriage of identical spa types.

The duration of the 82 episodes of intermittent carriage was estimated for 46 episodes of carriage. The duration of the other 36 episodes of intermittent carriage could not be estimated because the episode was not preceded, or followed, by a culture-negative swab. The median duration of the 46 episodes of intermittent *S. aureus* carriage was 4 weeks (IQR 2–6).

Only 6/188 (3%) nasal swabs submitted by persistent carriers did not culture *S. aureus*; the average carrier index was 0.96 (range 0.81—1) (Supplementary Figure). We observed three patterns of persistent *S. aureus* carriage — continuous carriage of a single *spa* type, an abrupt change from one *spa* type to another, and periods of co-carriage with two *spa* types. Fig. 1 shows that most persistent carriers (12/19, 63%), students A to M in Fig. 1, were colonised with only one *spa* type throughout the study period. This was confirmed by determining that the *spa* types of 12 randomly selected colonies, isolated from swabs collected at the midpoint of the study, were all identical to the *spa* types of the initial and final isolates.

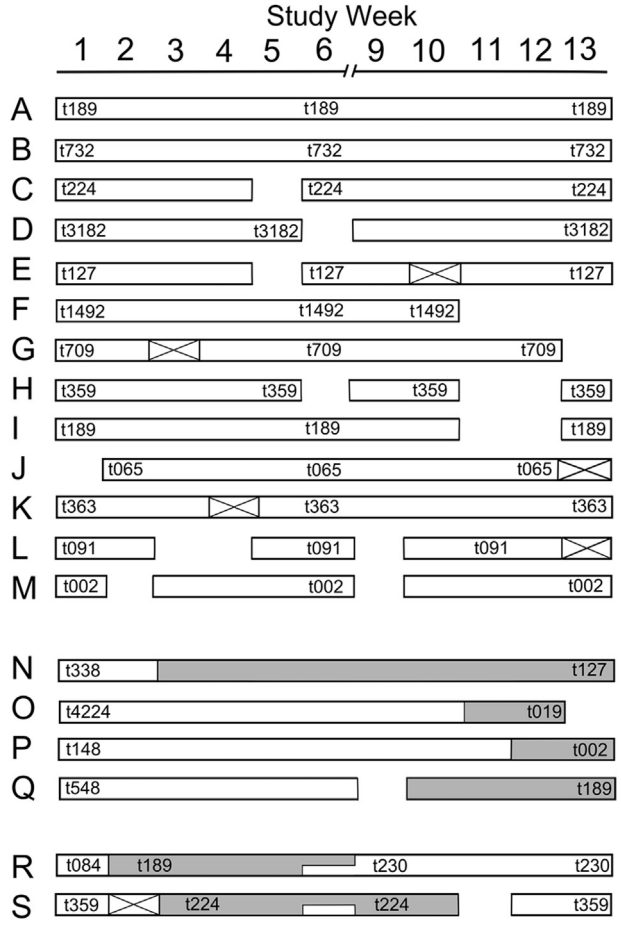

Figure 1 Patterns of nasal carriage in 19 persistent carriers (A—S) during a three month period that included a two-week study break between weeks 6 and 9. The numbers show the *spa* types of the isolated strains and the time that typing was performed; blank spaces indicate weeks in which no swab was submitted; crosses indicate the six swabs that did not grow *S. aureus*. Students A to M had persistent carriage of only one *spa* type. The *spa* types of isolates from students N—Q abruptly changed, confirmed by *spa* typing of 12 randomly selected colonies isolated from the swabs collected in the weeks before and after the *spa* type changed. Students R and S had periods of co-carriage of two different *spa* types. Student R had an abrupt change from spa type t084 to t189 at week 2 and then had two different *spa* types (t189, t1230) isolated from the sample collected in week 6. Student S had two different *spa* types (t224, t359) isolated from the swab provided in week 6.

A minority of persistent carriers (6/19, 32%), students N to S in Fig. 1, were colonised with more than one *spa* type during the study. Four students, N to Q in Fig. 1, had an abrupt change in their *spa* types during the study. In these students all twelve randomly selected colonies from the swabs collected during the last week of carriage with the first *spa* type had identical *spa* types as the initial isolate; and all twelve randomly selected colonies from the swabs collected during the first week of carriage with the second *spa* type had identical *spa* types as the final isolate. For example, in the case of student N, 12/12 colonies from week two were t338 and 12/12 colonies from week three were t127. In three of these four students the two *spa* types identified varied considerably (they were from unrelated *spa* clonal complexes); however the *spa* types obtained from student O, t4224 (08-16-16-02-25-17-24) and t019 (08-16-**02**-16-02-25-17-24), were closely related.

Two students, R and S in Fig. 1, were colonised with two or more *spa* types during the study, and had periods of co-carriage with two *spa* types. In student R the single colony tested at week one was t084, while at week two 12/12 colonies were t189, at week six 3/12 were t189 and 9/12 colonies were t230, at week nine, 12/12 colonies were t230, and at week twelve the single colony tested was t230. In student S the single colony tested at week one was t359, while at week three 12/12 colonies were t224, at week six 5/12 were t224 and 7/12 colonies were t359, at week ten 12/12 colonies were t224, at week twelve 12/12 colonies were t359, and at week 13 the single colony tested was t359. In student R the three *spa* types identified varied considerably (they were from unrelated *spa* clonal complexes); however the *spa* types obtained from student S: t359 (07-23-12-21-17-**34**-34-33-34) and t224 (07-23-12-21-17-34-33-34) were closely related.

Discussion

Our study used repeated weekly sampling, rather than sampling at more widely spaced intervals, to improve understanding of the short-term changes in nasal *S. aureus* carriage. The proportions of non, intermittent and persistent carriers were very similar to the proportions found in previous studies,[16] including those that used weekly nasal swabbing to characterise the carriage states of participants.[11,13] However, in contrast to the previous studies that used weekly nasal swabbing, we used *spa* typing to examine the turnover in the strains isolated from subjects with intermittent or persistent nasal carriage.

We found that turnover of *S. aureus* strains was frequent in intermittent carriers. Overall, the median interval between episodes of carriage was four weeks and the median duration of carriage was also four weeks. These results suggest that, during the course of one year, an intermittent carrier might expect to be briefly colonised by approximately six different *S. aureus* strains. The turnover of strains of *S. aureus* causing intermittent carriage may be even greater than this because some episodes of carriage may be sufficiently brief as to avoid detection by weekly swabbing.

We found that turnover of *S. aureus* strains was less frequent in persistent carriers. During the three month study period, one third of the persistent carriers changed *spa* types. By extrapolation, we expect that over the course of a year the other persistent carriers would also have been likely to change *spa* types and we estimate that the duration of carriage of a single *spa* type is in the order of six to nine months. This estimation is consistent with the findings of Miller et al.,[19] who observed that 50% of *S. aureus* carriers lost their carriage strain during a six month period; there was frequent replacement by another strain. Furthermore, our findings show that when a persistent carrier changed *spa* type, this occurred abruptly without an intervening non-colonised interval. Most changes in *spa* type were to unrelated *spa* types indicating acquisition of another strain; however we also identified two episodes of change to a closely related *spa* type, which raises the possibility of evolution of *S. aureus* within the nasal environment. If this were the case, then reversion back to the parental *spa* type (student S, week 11 on Fig. 1) is more consistent with genetic drift rather than with selection, which has been thought to be the main driver of *S. aureus* evolution in the nasal environment.[26]

In light of our findings, and those of other investigators,[1,11,13] we suggest that Fig. 2 represents *S. aureus* colonisation in most healthy adult human populations. Approximately 20% of the population are persistent carriers, who commonly carry a high density of *S. aureus* in their noses[11,13] for more than 90% of the time. The duration of colonisation by each strain commonly is many months and co-carriage of more than one strain is not uncommon. Approximately 30% of the population are intermittent carriers, who commonly carry a low density of *S. aureus* in their noses for approximately 50% of the time. The duration of colonisation by each strain is usually a few weeks and co-carriage of more than one strain is very rare. Persistent and intermittent carriers are the source of strains that are transmitted to and then colonise other persistent carriers or intermittent carriers. Persistent and intermittent carriers also contaminate the environment to place non-carriers at risk of *S. aureus* disease.

This representation draws attention to the multiple differences between persistent and intermittent carriers, in relation to the density of colonisation, the duration of each episode of colonisation by an individual strain, the duration of the interval between each episode of colonisation, and the prevalence of co-carriage by more than one strain.

It is likely that host characteristics are the principal determinants of a person's carriage state. Studies have

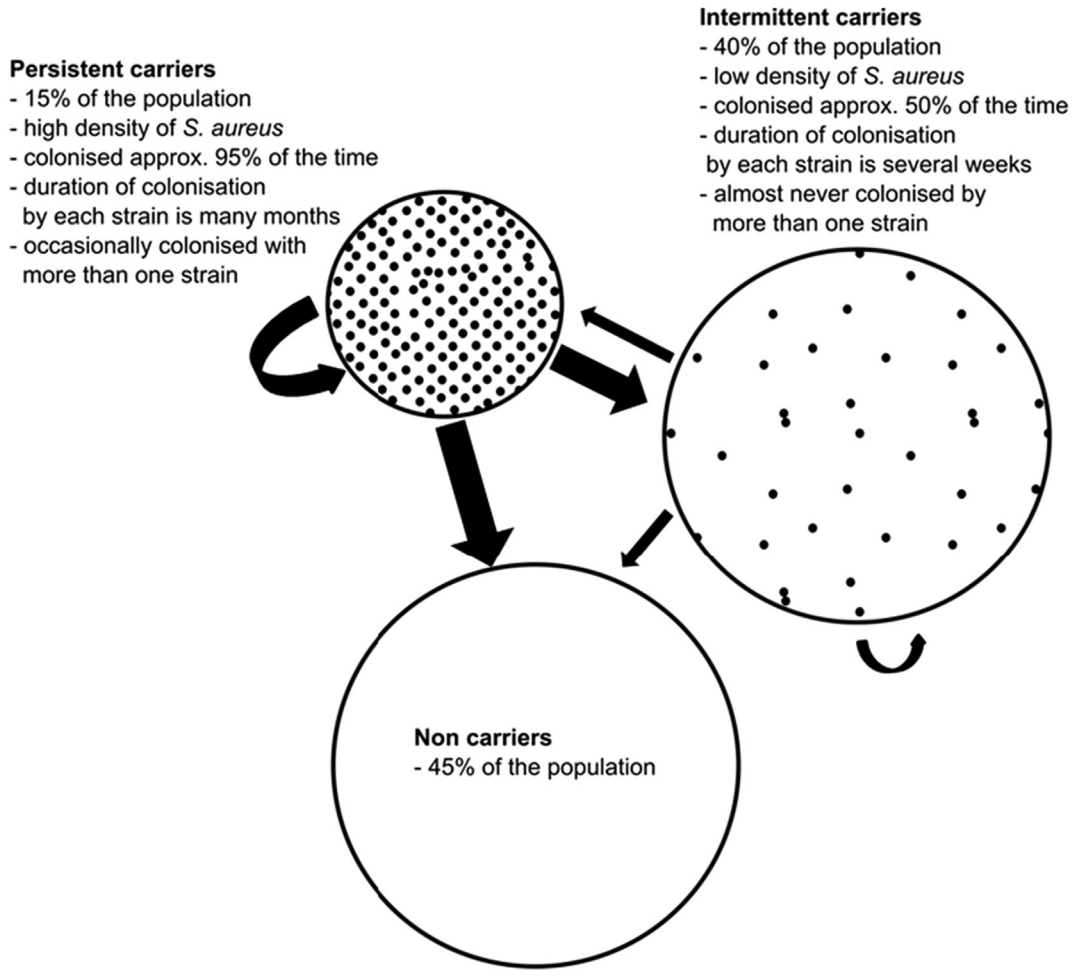

Figure 2 *S. aureus* carriage in healthy human populations.

shown that persistent nasal carriers differ immunologically and genetically from non-carriers.[12,27,28] These host characteristics also influence which strains are able to colonise the nose of persistent carriers. When persistent carriers were de-colonised and then re-challenged with a mixture of different *S. aureus* strains, the majority selected their own resident strain from the inoculum mixture.[12] This suggests that persistent carriers are not equally susceptible to colonisation by all *S. aureus* strains and are able to more effectively clear some *S. aureus* strains than other strains.

Our findings are consistent with those of Votintseva et al., who found that persistent carriers occasionally had transient episodes of co-carriage with two genetically distinct strains of *S. aureus*.[17] We found that persistent carriers most frequently had an abrupt change in *spa* types. The mechanism of the abrupt change is not known and might result from rapid complete clearance of the resident strain (e.g. by the host immune system, or by phage infection) followed by almost immediate colonisation with a new strain derived from an environmental source. This would require that environmental exposure is very frequent, in order for a new strain to opportunistically exploit an empty niche within a matter of days. It is more likely that abrupt change in persistent carriage results from rapid proliferation of either a new *S. aureus* strain, or a strain already present in very low frequency, which replaces the resident strain.

The rapid turnover of *S. aureus* strains in intermittent carriers, and the less frequent turnover of *S. aureus* strains in persistent carriers, was in marked contrast to our findings in the remaining 43% of subjects in our study who never had *S. aureus* isolated from a nasal swab. It is hard to believe that these non-carriers were not equally exposed to the sources of *S. aureus* that frequently resulted in colonisation by new strains in the intermittent carriers and persistent carriers. Although the proportion of students who met our definition of non-carrier (no positive swabs) might have declined by a small amount over a longer period of study, previous research (with less frequent swabbing) has shown that a high proportion of people remain non-carriers over long time periods.[19] Our findings support the hypothesis that non-carriers form a distinct group that are in some way resistant to *S. aureus* colonisation.[12]

Our study was performed in healthy students, who are not representative of the total population; however, few had any recent interaction with the healthcare system, which might have impacted the results. More than 100 subjects provided at least eight out of a possible eleven weekly nasal swabs during a 13 week period and we used *spa* typing to identify changes in the strains colonising individuals and carefully studied the colonising strains to look for co-carriage. Potential limitations of our study were our reliance on students to collect their own nasal swabs and our decision not to culture the swabs in multiple media.[29] However, self-swabbing has been used successfully before,[19,23,24] and the proportion of nasal carriers that we identified (54%) was similar to that found in other longitudinal studies.[11,13] Finally, it is possible that we missed some episodes of mixed carriage, as we did not *spa* type multiple colonies from all of the swabs provided by persistent carriers. It is also possible that *spa* typing more than 12 colonies from a nasal swab culture, would have allowed increased detection of mixed carriage, if one of the strains was at very low density in the nose.

Conflicts of interest

None.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jinf.2015.12.010.

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