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CHARACTERISATION AND TYPING OF MICRO-ORGANISMS

Typing of *Staphylococcus aureus* colonising human nasal carriers by pulsed-field gel electrophoresis

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Summary. Colonisation by *Staphylococcus aureus* in the nares of 120 outpatients and 63 healthy adults was studied for *c.* 2 years. Two states of carriage of *S. aureus* were confirmed: persistent carriage and persistent non-carriage. The states of carriage and non-carriage were quite stable and > 60 % of the population of any of the study groups were stable non-carriers. The results of typing the strains isolated from the same individuals at different times with DNA fingerprinting by digestion with *Sma*I enzyme showed that all the stable carriers were persistently infected with the same strain and that changes in the strain seldom occurred.

Introduction

The bacterial flora of the skin and mucosa includes potential pathogens such as *Staphylococcus aureus*. The nares are frequent sites of colonisation by *S. aureus* and 10–40 % of the population carries this organism.[1–4] Long-term follow-up studies on the isolation of *S. aureus* from healthy persons or outpatients suggested that the carrier state was stable and subjects could be divided into three classes: persistent carriers, persistent non-carriers and transient or intermittent carriers.[2,4–7] Despite the many epidemiological analyses of the carriage state it is still not clear whether the same strain stably colonises the nares of persistent carriers or whether they are infected by different strains repeatedly. To answer this question it is necessary to follow the changes in the types of the strains isolated from the same person over a long time.

The electrophoresis pattern of chromosomal DNA digested with *Sma*I was reported recently to be superior to other typing methods such as phage typing or coagulase typing for *S. aureus*.[8–12] Ichiyama *et al.* identified 31 restriction patterns amongst 111 isolates of methicillin-resistant *S. aureus* by their *Sma*I digestion patterns.[9]

In this study the changes in the types of *S. aureus* colonising the human nasal cavity were followed for > 1 year.

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Materials and methods

Study population

The study population comprised 183 individuals. They included 120 outpatients of the clinic for rheumatoid arthritis in Fukuoka Medical Center, Japan, and 63 healthy adult volunteers. The healthy adults comprised 51 students and 12 healthy adults working in a laboratory. All gave informed consent to this experimental programme.

Collection of specimens

Specimens for the isolation of *S. aureus* were obtained from the right nares with sterile cotton swabs moistened in sterile physiological saline, once or twice a month for *c.* 1 year (April 1990–May 1991) for the healthy volunteers, and once every 4–6 months for 2 years (April 1990–May 1992) for the outpatients.

Media and identification of the strains

The swabs were immediately streaked on *S. aureus* Selection Medium No. 110 (Eiken Kagaku Co. Ltd, Tokyo, Japan). After incubation for 48 h at 37°C, suspected colonies of *S. aureus* were subcultured on Mannitol Salt Agar (Eiken Kagaku Co.). Strains that fermented mannitol were tested for coagulase activity (tube test with heparinised normal rabbit plasma). Strains that produced coagulase were identified as *S. aureus*.[13] These strains were stored in soft nutrient agar.

Table I. Reproducibility of isolation of *S. aureus* from nares with wet cotton swabs

Experiment no.	Side of nostril	Colony counts from subjects		
		L4	L5	L10
1	L	660	30	0
	R	382	55	0
2	L	760	193	0
	R	87	30	0
3	L	330	470	0
	R	45	32	0

Table II. Rate of isolation of *S. aureus* in different study groups

Group	Month	Number tested	Positive (%)
Students	Jan. 1991	51	11 (22)
	April 1991	51	11 (22)
	May 1991	51	11 (22)
Outpatients	April 1990	120	36 (30)
	Oct. 1990	120	43 (36)
	May 1991	120	41 (34)

Table III. Patterns of isolation of *S. aureus* at different times (a)

Study group	Number of persons	Time of isolation		
		Jan. 1991	April 1991	May 1991
Student	35	−	−	−
	6	+	+	+
	1	+	+	−
	1	+	−	+
	2	−	+	+
	3	+	−	−
	2	−	+	−
	1	−	−	+
Total	51			

(b)

Study group	Number of persons	Time of isolation		
		April 1990	Oct. 1990	May 1991
Outpatient	63	−	−	−
	25	+	+	+
	4	+	+	−
	3	+	−	+
	8	−	+	+
	5	−	−	+
	6	−	+	−
	6	+	−	−
Total	120			

Preparation of chromosomal DNA

Chromosomal DNA of *S. aureus* was prepared by the method of Weil and McClelland.[14] The cells from a late log phase culture were collected by centrifugation. After being washed with TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7·6) the bacteria were suspended in 100 mM EDTA buffer (pH 8·0) at a concentration of 10^9 cells/ml. The suspension was mixed with melted low-melting-point agarose L (Wako Pure Chemical Industries Ltd, Osaka, Japan). After solidification the plugs were incubated in 100 mM EDTA buffer containing lysozyme 1 mg/ml and lysostaphin 0·5 mg/ml for 6–8 h at 37°C. Then the plugs were incubated in 250 mM EDTA containing proteinase K 1 mg/ml and SDS 1% overnight at 50°C.

Pulsed-field gel electrophoresis

The DNA extracted from each agarose block prepared as described above was digested with enzymes *Sma*I or *Eco*52II (Toyobo Co. Ltd, Osaka, Japan). The block was then loaded on the top of an agarose 1% gel for electrophoresis in a PFGE apparatus (type BR-550, Biocraft Corp., Tokyo, Japan). The pulse time was 10 s for 3 h, 20 s for the next 10 h, 30 s for the next 10 h and 40 s for the final 4 h. Chromosomal DNA of *Saccharomyces cerevisiae* strain YNN 295 (Clontech Laboratory, USA) was used as a DNA size marker.

Results

Efficiency of the isolation of S. aureus by a cotton swab

The isolation of *S. aureus* from the nares with a wet cotton swab was reproducible (table I). *S. aureus* was isolated from both nares of subjects L4 and L5 on three occasions, although the numbers of colonies varied. *S. aureus* was not isolated from subject L10 throughout the experimental period.

Rate of isolation of S. aureus from the nasal cavity

The rates of isolation of *S. aureus* from the nasal cavities of 171 individuals at different times between April 1990 and May 1991 are shown in table II. The rate varied from 22% to 36% depending on the study population. In all cases, staphylococci other than *S. aureus* were always isolated.

To confirm the existence of the carrier state, the pattern of isolation was further analysed in detail (table III). Among the 51 students, 35 were always negative and six were always positive during the study period (Jan. 1991–May 1991; table IIIa). Thus 12% of the population in this group seemed to be stable nasal carriers of *S. aureus*. During this period the carrier state changed from positive to negative in only a small fraction of the study population. The same phenomenon was found in the larger outpatient group followed over a longer period from April 1990 to May 1991, (table IIIb). Among the 120 subjects, 25 were always positive for *S. aureus* and 63 were always negative. These results suggest that the carriage of *S. aureus* in the human nasal cavity is stable and that a large proportion of the population are non-carriers of *S. aureus*.

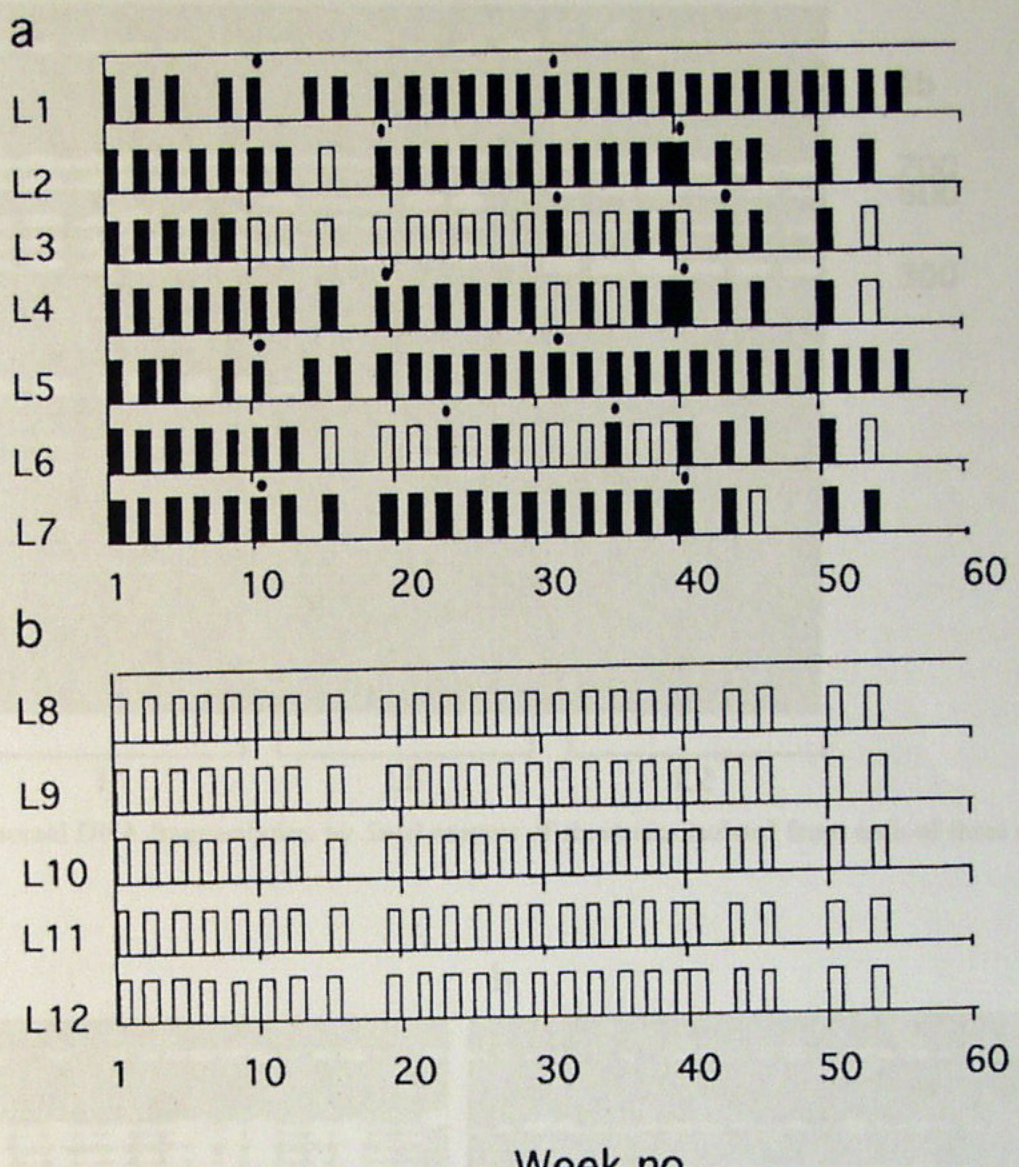

Fig. 1. Isolation of *S. aureus* from healthy volunteers over a 1-year period. **a**, persistent carriers. **b**, persistent non-carriers. The identification numbers of the subjects are shown at the left of the figure. Each bar marks the date of attempted isolation of *S. aureus*: □, no isolation; ■, isolation. A dot over the bar indicates that the strain isolated on that date was used for DNA typing.

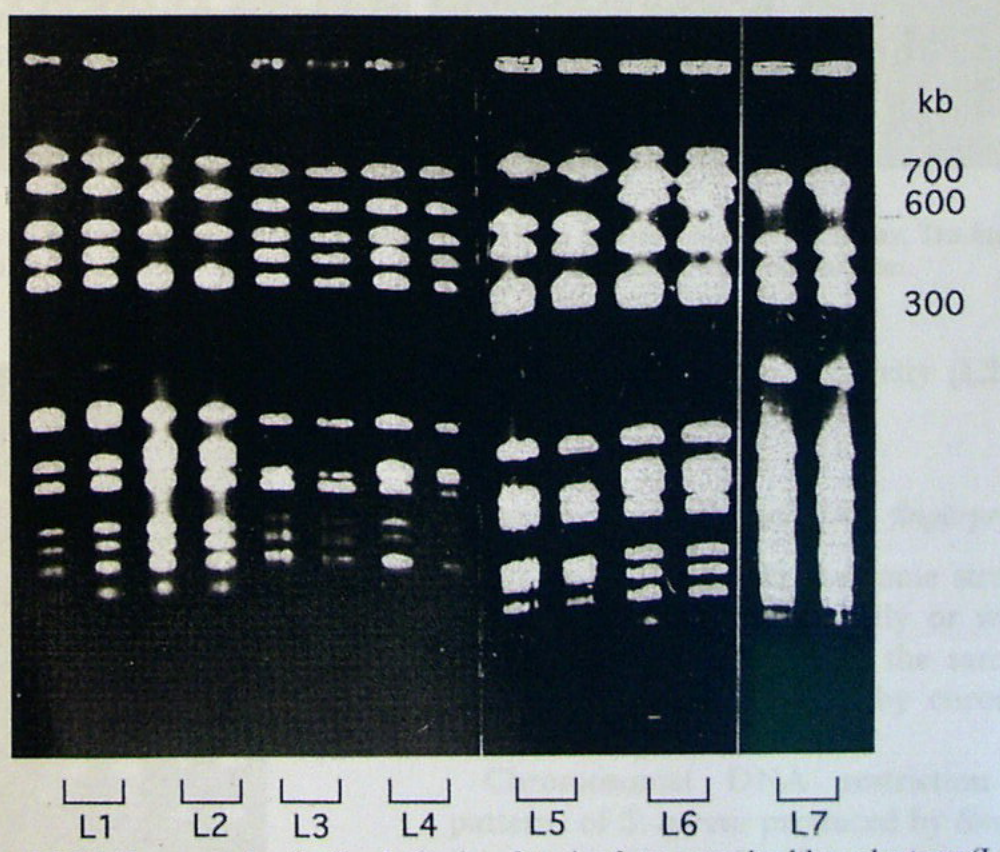

Fig. 2. Pattern of chromosomal DNA fragmentation by *Sma*I of pairs of strains from seven healthy volunteers (L1–L7). The two strains of each pair were isolated on different days separated by at least 3 months. The molecular sizes of the bands are shown on the right.

Long-term follow-up study of healthy volunteers

To follow the long-term carriage of *S. aureus* attempts were made to isolate *S. aureus* consecutively from the nares of 12 healthy volunteers for *c.* 1 year and the results are shown in the figure. It is clear from these results that there are two basic types of isolation pattern. One is the carrier state (fig. 1a) and the other is the non-carrier state (fig. 1b). Some of the carriers became negative for *S. aureus* for short periods but

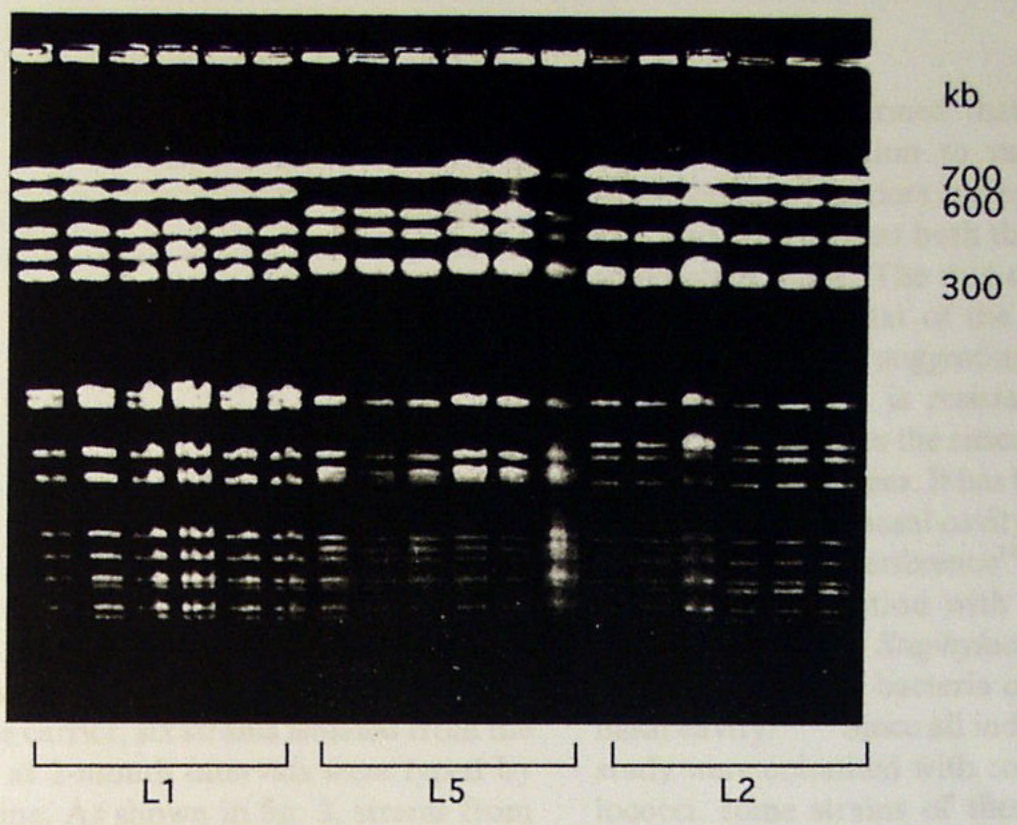

Fig. 3. The pattern of chromosomal DNA fragmentation by *Sma*I enzyme of six strains isolated from each of three carriers on 6 different days in 1 year.

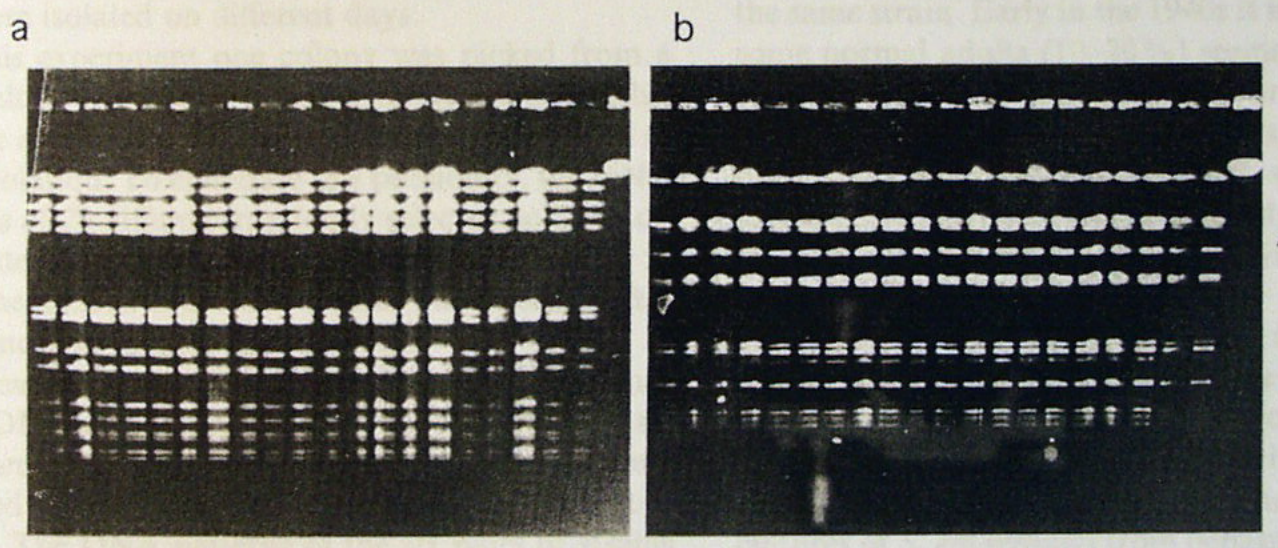

Fig. 4. The pattern of chromosomal DNA fingerprinting of 20 strains isolated from one person on the same day. The fingerprinting patterns were created by enzymes *Sma*I (**a**) and *Eco*52II (**b**). The 20 strains showed the same patterns with two enzymes.

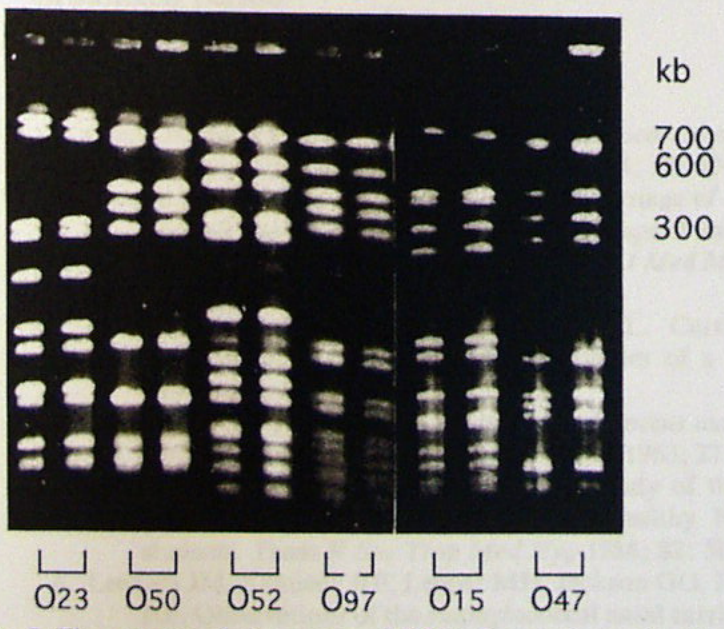

Fig. 5. The pattern of chromosomal DNA fingerprinting by *Sma*I enzyme of the pairs of strains isolated from six outpatients. The patients are indicated by the numbers at the bottom of the figure and their isolation patterns are shown in table IV. The two pairs of strain were isolated on days separated by *c.* $\geqslant$ 6 months.

they soon returned to positivity (L2, L3 and L6 in fig. 1a).

Typing by chromosomal DNA fingerprinting

To find out whether the same strain of *S. aureus* colonises a carrier persistently or whether different strains occur, isolates from the same individual at different times were typed by chromosomal DNA fingerprinting.

Chromosomal DNA restriction fragmentation patterns of *S. aureus* produced by *Sma*I digestion fall into two groups and each group contains five to eight DNA fragments (fig. 2). Variations in the size and the number of the fragments in each group were found in the strains tested. The patterns produced by *Sma*I enzyme were similar to those reported by Ichiyama *et al.*[9]

Pairs of strains isolated from the same individuals in

Table IV. Patterns of isolation of *S. aureus* from nares of outpatients studied between April 1990 and May 1991

Patient no.	Time of isolation		
	April 1990	Oct. 1990	May 1991
O23	+	+	+
O50	+	+	+
O52	+	+	+
O97	+	+	+
O15	–	+	+
O47	–	+	+

the carrier group showed the same pattern (fig. 2). To confirm that the same clone of *S. aureus* continuously colonises the same carrier, six strains isolated from the same individuals at 2-month intervals were typed by DNA fingerprinting. As shown in fig. 3, strains from three subjects (L1, L2 and L5) showed their own fingerprint patterns, and the strains isolated from one individual showed the same DNA pattern even though they were isolated on different days.

In this experiment one colony was picked from a plate culture of one individual. Therefore it is possible that the same clone had been unexpectedly picked at every isolation. To eliminate this possibility, the DNA patterns of 20 different randomly selected colonies on one plate from one person were tested. As shown in fig. 4, the pattern of these 20 strains was the same after *Sma*I and also in *Eco*52II digestion.

The same experiment was performed on chromosomal DNA isolated from pairs of strains from the six outpatients listed in table III and the same results were obtained as had been found with the laboratory staff (fig. 5). The DNA patterns of the six pairs of strains isolated in October 1990 and in May 1991 (table IV) were the same. The results show that each person was colonised with a single clone of *S. aureus* over a prolonged period.

Discussion

This study confirmed that there are two types of individual in relation to nasal carriage of *S. aureus*—persistent carriers and persistent non-carriers[2,4,7] and also showed that both the carrier and non-carrier states were stable. The rate of persistent non-carriers was higher than that of the persistent carriers in all study populations, suggesting that a large proportion of the population is resistant to infection with *S. aureus*. What causes the susceptibility of the colonised individuals is not clear. It has been proposed that minor deformities of the nasal cavity,[15] genetic influences[16,17] and bacterial interference[18,19] are involved. It is possible that infection with *S. aureus* is blocked by resident strains of *Staphylococcus* spp. other than *S. aureus* or by other bacteria of the normal flora of the nasal cavity.[18,19] Since all individuals examined in this study were colonised with coagulase-negative staphylococci, some strains of these bacteria might play a role in bacterial interference.

Typing by the DNA fingerprinting method showed that isolates of *S. aureus* from a persistent carrier were the same strain. Early in the 1940s it was reported that some normal adults (10–20%) seemed never to yield staphylococci from the nares even on repeated swabbing, some people could harbour a single phage type of *S. aureus* for several years,[20] and intermittent carriers often carried different phage types.[21] It is likely that in carriers only one predominant strain persistently colonises the nasal cavity and changes in strains rarely occur. It is possible that some mechanism of bacterial interference inhibits the colonisation of *S. aureus*, perhaps involving the production of bacteriocins or phages. We are now investigating the production of interfering or bactericidal substances in cultures of *S. epidermidis* from persistent non-carriers.

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