BACTERIAL PATHOGENICITY

Coagulase and protein A polymorphisms do not contribute to persistence of nasal colonisation by *Staphylococcus aureus*

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The nasal carriage rate of *Staphylococcus aureus* was examined in a longitudinal study of 31 healthy Danish volunteers. Each person was classified as persistent (>8 positive cultures from 10 examinations), an intermittent carrier (50–80% positive cultures) or an occasional carrier (positive cultures on 10–40% of occasions only). One hundred and twenty strains from these persons were subjected to phage typing and random amplification of polymorphic DNA (RAPD) analysis. Phage and RAPD typing were in close agreement. RAPD confirmed the spread of a particular *S. aureus* clone (phage type 95) throughout Denmark. However, no common genotype or phenotype characteristics of *S. aureus* that could separate persistent from intermittent or incidental colonisers were identified. The immunoglobulin binding protein A and the prothrombin binding coagulase protein are both putative *S. aureus* virulence or defence factors. Analysis of polymorphisms in the variable repeat regions in the genes for these proteins showed no correlation between the number of repeat units and, consequently, the protein structure with the ability of strains to persist in the human nasal mucosa. The amount of protein A, detectable by its IgG binding activity, appeared not to be correlated to persistence of carriage. Thus protein A and coagulase gene polymorphisms do not seem to play a significant role in the propensity of *S. aureus* to colonise human nasal epithelium. Furthermore, based on the genetic heterogeneity encountered among the *S. aureus* strains it is suggested that within the current study population, no single clonal lineage of *S. aureus* has increased capability to colonise the human nasal epithelium.

Introduction

Nasal carriage of *Staphylococcus aureus* can be a precursor of severe post-operative complications in surgical patients [1]. For example, in patients who undergo thoracic surgery, a subsequent surgical wound infection with *S. aureus* can be a reason for a prolonged stay in hospital, as well as increased morbidity and even mortality [2, 3]. To prevent post-operative complications, patients may be treated pre-operatively by nasal application of antibiotic-containing ointments. However, examination of the ways in which *S. aureus* colonises the nasal epithelium may produce new approaches to the elimination of colonisation. How it successfully colonises human epithelium is still largely unknown, although many factors relating to adhesion have been described previously [4–6]. To

evaluate new therapeutic approaches for the elimination of nasal carriage, the molecular basis of persistent colonisation needs to be resolved.

In the present study individuals who were persistently or intermittently colonised with *S. aureus* were identified by longitudinal microbiological follow-up. The strains were typed to identify common genotypic and phenotypic denominators. Genetic polymorphisms in two genes encoding proteins that protrude from the bacterial surface and are thought to play a role in the spread or pathogenicity of *S. aureus*, or both, were determined to elucidate the role of these proteins in epithelial colonisation.

Materials and methods

Human volunteers

The initial study population comprised 86 female and 18 male healthy, normal volunteers [7]. They were all

employed at the Statens Seruminstitut (Copenhagen, Denmark), in different departments and buildings. Their median age was 46 (range 23–69) years. Serial nasal swabs were collected over a 19-month period (January 1992–August 1993) by one investigator and by a standardised technique. The left and right anterior nares were swabbed with sterile, dry cotton-wool swabs (Dansu A/S, Ganlose, Denmark) [8]. The entire anterior nares were swabbed by rubbing twice around the nostril, applying an even and uninterrupted pressure. When cultures were consistently positive or positive in nine out of 10 assays, the individual was identified as a persistent carrier (carriage index 10 or 9). When 8, 7 or 6 cultures out of 10 were positive, the individual was described as an intermittent carrier. Lower scores indicated sporadic carriage.

Cultivation of S. aureus from nasal swabs

Swabs were inoculated directly on to 5% blood agar and mannitol salt agar [6]. The swabs were then incubated in broth enrichment medium with NaCl 6.5% at 37°C for 18 h or in Stuart's transport medium for 7 days at 4°C [9]. All solid media were incubated at 37°C for 16 h, after which plates were placed at room temperature and left for up to 6 days.

Identification of S. aureus

Colonies were selected on the basis of morphological characteristics, checked by gram-stained films and tested for catalase and coagulase production. Coagulase production was assayed by the citrate-plasma tube technique [8].

Phage typing

The method of Blair and Williams was used [10]. The phages comprised the current international set with two additional local Danish phages. Bacteria were assayed by application of routine test dilutions (RTD) of the phages. When strains appeared to be non-typable under these conditions, 100 × RTD and 1000 × RTD were also tested. Subdivision into groups was as described by Parker [11].

RAPD analysis

Bacteria were grown in suspension in brain heart infusion (BHI) broth for 18 h at 37°C. A volume of c. 100 µl of a bacterial pellet was suspended in 150 µl of 25 mM Tris-HCl, pH 8.0, 50 mM glucose and 10 mM EDTA. Lysostaphin (75 µl of a 100 µl/ml solution) was added and the mixture was incubated at 37°C for 1 h. Spheroplasts were lysed by the addition of 1 ml of 4 M guanidinium isothiocyanate, 50 mM Tris-HCl, pH 6.4, 3 mM EDTA, Triton X-100 1% w/w [12]. To immobilise and purify the DNA, 50 µl of a Celite suspension (0.2 g/ml; Janssen Pharmaceuticals, Beerse, Belgium) was added. The entire mixture was shaken

for 15 s and incubated at room temperature for 10 min. The pellet was washed once with 1 ml of lysis buffer, twice with lysis buffer without EDTA and Trinton X-100, twice with ethanol 70% in water and finally once with acetone. Between 100 and 400 µl of 10 mM Tris-HCl, pH 8.0, 1 mM EDTA were added and DNA was eluted by incubation at 56°C for 10 min. The supernate containing the DNA was separated from the Celite by centrifugation. Stock solutions of bacterial DNA were adjusted to a concentration of 5 ng/10 µl and stored at –20°C.

PCR was performed in a buffer system containing 10 mM Tris-HCl, pH 9.0, 50 mM KCl, 2.5 mM MgCl₂, gelatin 0.01% Triton X-100 0.1%, 0.2 mM of the respective deoxynucleotide triphosphates, 50 pmoles of primer and 0.2 units of the Tth polymerase (Supertaq; HT Biotechnology, Cambridge), to which DNA was added (5 ng for each amplification). The PCR mixtures were overlaid with 100 µl of mineral oil. Cycling was performed in Biomed PCR machine (Model 60) and consisted of the following steps: predenaturation at 94°C for 4 min and 35 cycles of 1 min at 94°C, 1 min at 25°C and 2 min at 74°C. The primers used to discriminate *S. aureus* strains were 1 (GGTTGGGTGAGAATTCACG), 7 (GTGGATGCGA) and ERIC2 (AAGTAAGTGACTGGGGTGAGCG) [13–15].

Electrophoresis

Amplification products were separated by electrophoresis in agarose 1% gels, 5 mm thick (Hispanagar, Sphaero Q, Leiden, the Netherlands). Gels were run in 0.5 × TBE at a constant current of 100 mA for 3 h. Before electrophoresis, samples were mixed with a 5 × concentrated layer mix consisting of glycerol 50% in water and bromophenol blue 0.8 mg/ml; 35 µl of the amplified material were loaded on to the gel and a mol. wt marker was run in parallel with the RAPD samples. Gels were stained before electrophoresis by addition of 10 µl of ethidium bromide (10 mg/ml) to a total volume of 300 ml of 0.5 × TBE. Gels were photographed with a Polaroid MP4 Landcamera and Polaroid 57 High Speed films; the exposure time was 0.125–0.25 s (diaphragm F5.6). Banding patterns visualised in this manner were indexed by Roman lettering. Even single band differences led to the definition of a novel type. This is based on the percentile effect of a single band difference between banding patterns comprising 6–15 different DNA fragments. In this way all the bacterial isolates were identified by a three-letter code.

Protein A gene PCR

Length polymorphisms in the staphylococcal protein A gene were determined by PCR essentially as described before [16]. Briefly, the repetitive region within the gene was amplified with oligonucleotide primers

with the following DNA sequences: 5'-TGTAACGACGGCCAGTGCTAAAAAGCTAACGATGC-3' and 5'-CAGGAAACAGCTATGACCCACCAAATACAGTTGTTACC-3'. After PCR, DNA was cleaved with the restriction endonuclease *Rsa*I (Boehringer Mannheim, Germany) and restriction fragment length polymorphisms (RFLP) were determined by electrophoresis in Nusieve GTG agarose 3% gels (Biozymn, the Netherlands) run in $0.5 \times$ TBE. The number of repetitive units present in the genes' variable region was estimated in comparison with mol.wt markers (100 bp ladder; Pharmacia, Gouda, The Netherlands).

Coagulase gene PCR

The analysis was aimed at the 3' end of the coagulase gene. Amplification was done with primers COAG2 and COAG3 [17]. The DNA sequences of these primers are 5'-CGAGACCAAGATTCAACAAG-3' and 5'-AAAGAAAACCACTCACATCA-3'. DNA (5 ng) was amplified by 40 cycles of 1 min at 95°C, 1 min at 52°C and 1 min at 74°C, after a predenaturation period of 4 min at 95°C. The PCR mixture was as described above. The amplification product (10 µl) was digested with the restriction endonuclease *Alu*I (Boehringer Mannheim, Germany). Restriction fragments were analysed as described above for the protein A gene analysis. Digests were analysed with respect to the overall number of 81-bp repeats detectable and in regard to the actual RFLP pattern, in comparison with a 100-bp mol. wt marker (Pharmacia).

Protein A assay by IgG binding

Bacteria were grown overnight on 5% blood agar (Statens Seruminstitut, Copenhagen, Denmark). Between five and 10 colonies were suspended in phosphate-buffered saline, pH 7.4 (PBS), and the optical density (540 nm) was adjusted to 0.4. This represented a bacterial density of *c.* $(2-3) \times 10^8$ cfu/ml. Bacteria were diluted to working concentrations of 5×10^6 cfu/ml of PBS; 100 µl of this suspension were used for coating Nunc Immunoplates (A/S Nunc, Roskilde, Denmark); after 1 h at 20°C, the plates were washed three times with PBS. Remaining binding sites were blocked by incubation for 1 h at 20°C and overnight incubation at 4°C with 100 µl of PBS containing Triton X-100 0.1% (PBST). After three washes, 100 µl of peroxidase-conjugated rabbit anti-mouse immunoglobulins (DAKO, Glostrup, Denmark) diluted in PBST were applied to each well and incubated for 1 h at 20°C. After five washes in PBST the plates were stained for 30 min at room temperature in the dark with citric acid buffer (pH 5.0) containing OPD (Kem-En-Tec, Copenhagen, Denmark) and hydrogen peroxide 3%. The colour reaction was stopped by the addition of 100 µl of 1 N H₂SO₄. All bacterial strains were tested in triplicate and the reference strain *S. aureus* Cowan I was included on each microtitration plate. The IgG binding was expressed as the ratio of the mean OD

measured for the strain under investigation and that determined for *S. aureus* Cowan I strain.

Results

Carrier rates

Of the 31 individuals enrolled in the study, six had *S. aureus* cultured from their nares on every occasion. An equal number were culture-positive in nine out of 10 nasal swabs. The group comprised 12 persistent, nine intermittent and 10 occasional carriers. The general carrier index varied from three to 10 in this group (Table 1). The length of the screening period varied from 8 to 31 months; no major differences were observed between data obtained for left and right nostrils.

Phage types and RAPD analysis

Results of both phage typing and RAPD analysis are summarised in Table 1. An example of the RAPD data is shown in Fig. 1. The results of both typing strategies were very similar, e.g., RAPD type BBB was identical to phage type 95; this occurs relatively frequently. Overall, RAPD identified 25 different genotypes among these *S. aureus* strains. When the number of types encountered in a given individual was plotted against the carriage index, it is seen that in those who were colonised intermittently or occasionally, multiple types were encountered (Table 2). It is also clear that phage typing identified larger numbers of phagovars than RAPD analysis identified genotypes. This may relate to different phage typing results as a consequence of differences in cultivation or bacterial storage [14]. When the RAPD type was plotted against the carriage rate (Fig. 2) genotype BBB was shown to be prevalent. Also, types AAA, CCC, DDD, EEE, JIH, MLJ, PNC and OOL were encountered in several volunteers.

Variability in coagulase and protein A genes

Variation in the coagulase gene could be demonstrated at two levels. First, restriction fragment length polymorphism (RFLP) was evident as 12 different banding patterns were observed (Fig. 3). The overall number of repeats was assessed also: this number varied from five to 15 (Table 1). Fig. 4 shows the comparable RFLP results obtained with the analysis of the protein A gene. The number of repeat units as estimated by gel electrophoresis varied from three to 11. There appeared to be a clear overlap between the coagulase and protein A typing data. The numbers of repeats detected in both genes were not correlated. A large number of repeats in the protein A gene was found to be accompanied by variable repeat numbers in the coagulase gene and *vice versa*. When the RAPD results and data obtained by coagulase and protein A gene mapping were compared, it was clear that, as a single technique, RAPD provided optimal resolution: 25 distinct genotypes were identi-

Table 1. Carriage characteristics and phenotypic and molecular typing data of *S. aureus* strains

Strain no.	Patient no.	Carriage index	Nostril	Date of isolation	Phage type	RAPD type	Number of Protein A repeats	Relative amount of protein A	Coagulase gene type
Persistent carriers									
1	17	10	R	07-01-1992	793	AAA	7	0	12A
2	17	10	L	31-08-1992	793	AAA	7	0	12A
3	17	10	R	14-02-1994	811	BBB	9	0	14B
4	17	10	R	02-05-1994	313	BBB	9	1	14B
5	17	10	R	15-08-1994	793	AAA	7	0	12A
6	17	10	L	15-08-1994	583	AAA	7	0	12A
7	19	10	R	07-01-1992	811	BBB	9	1	14B
8	19	10	L	27-04-1992	692	BBB	9	1	14B
9	19	10	R	31-08-1992	692	BBB	9	1	14B
10	19	10	L	19-10-1992	191	BBB	9	1	14B
11	19	10	R	09-08-1993	811	BBB	9	1	14B
12	19	10	R	14-02-1994	811	BBB	9	1	14B
13	25	10	R	07-01-1992	232	CCC	10	93	9C
14	25	10	R	24-02-1992	292	CCC	10	137	9C
15	25	10	R	18-04-1993	232	CCC	10	99	9C
16	25	10	L	09-08-1993	222	CCC	10	131	9C
17	25	10	L	02-05-1994	232	CCC	10	134	9C
18	25	10	R	15-08-1994	232	CCC	10	115	9C
19	51	10	L	24-02-1992	641	DDD	4	92	9C
20	51	10	L	09-03-1992	641	DDD	4	64	9C
21	51	10	R	27-04-1992	641	DDD	4	80	9C
22	51	10	L	18-04-1993	641	DDD	4	83	9C
23	51	10	R	14-02-1994	641	DDD	4	87	9C
24	51	10	R	02-05-1994	641	DDD	4	62	9C
25	68	10	L	24-02-1992	191	EEE	7	93	8D
26	68	10	R	09-03-1992	191	EEE	7	106	8D
27	68	10	R	27-04-1992	191	EEE	7	101	8D
28	68	10	R	25-06-1992	192	EEE	7	89	8D
29	68	10	R	31-08-1992	191	EEE	7	93	8D
30	68	10	R	19-10-1992	581	EEE	7	93	8D
31	120	10	L	19-10-1992	692	BBB	5	21	14B
32	120	10	R	06-01-1993	692	BBB	5	16	14B
33	120	10	R	18-04-1993	811	BBB	5	15	14B
34	120	10	L	01-11-1993	811	BBB	5	17	14B
35	120	10	L	02-05-1994	811	BBB	5	22	14B
36	120	10	L	15-08-1994	811	BBB	5	16	14B
37	26	9	R	07-01-1992	292	FFC	6	11	8D
38	26	9	R	24-02-1992	232	FFC	6	15	8D
39	26	9	L	19-10-1992	232	FFC	6	10	8D
40	26	9	R	01-11-1993	192	FFC	7	58	8D
41	26	9	L	14-02-1994	192	FFC	6	27	8D
42	26	9	R	02-05-1994	232	FFC	7	48	8D
43	53	9	L	24-02-1992	793	GGF	—	0	5E
44	53	9	L	09-03-1992	641	DDD	4	94	9C
45	53	9	R	31-08-1992	793	AAA	7	0	12A
46	53	9	R	09-08-1993	542	GGG	10	12	10F
47	53	9	R	01-11-1993	693	GGG	10	21	10F
48	53	9	L	15-08-1994	542	GGG	9	12	10F
49	58	9	R	24-02-1992	191	EEE	9	93	10G
50	58	9	R	19-10-1992	191	EEE	9	95	10G
51	58	9	R	01-11-1993	191	EEE	9	98	10G
52	78	9	R	24-02-1992	213	IHC	7	86	9H
53	78	9	R	06-01-1993	812	IHC	7	111	9H
54	84	9	R	09-03-1992	692	JIH	8	7	15I
55	84	9	R	18-04-1993	692	JIH	7	10	15I
56	84	9	R	01-11-1993	811	BBB	5	24	14B
57	84	9	L	15-08-1994	292	KJI	9	74	9C
58	126	9	R	19-10-1992	793	LKC	8	92	11N
59	126	9	R	18-04-1993	812	BBB	7	96	14B
60	126	9	L	15-08-1994	811	BBB	7	104	14B
Intermittent carriers									
61	85	8	R	24-02-1992	311	JIH	7	66	15I
62	85	8	L	09-03-1992	812	BBB	8	0	14B
63	85	8	L	06-01-1993	232	BBB	8	0	14B
64	85	8	L	15-08-1994	362	JIH	9	0	15I
65	122	7	L	06-01-1993	692	JIH	9	0	15I
66	122	7	L	01-11-1993	311	JIH	9	31	15I
67	122	7	R	14-02-1994	332	JIH	9	23	15I
68	122	7	L	14-02-1994	311	JIH	9	25	15I
69	2	6	L	25-06-1992	813	BBB	9	1	14B
70	2	6	R	19-10-1992	691	BBB	9	1	14B
71	2	6	L	18-04-1993	793	AAA	7	0	12A
72	2	6	R	14-02-1994	811	BBB	9	1	14B

Table 1. (continued)

Strain no.	Patient no.	Carriage index	Nostril	Date of isolation	Phage type	RAPD type	Number of Protein A repeats	Relative amount of protein A	Coagulase gene type
73	9	6	R	25-06-1992	811	BBB	5	31	14B
74	9	6	L	19-10-1992	292	CCC	7	72	9C
75	9	6	L	01-11-1993	233	CCC	7	72	9C
76	9	6	R	15-08-1994	191	MLJ	5	41	—
77	22	6	R	25-06-1992	692	MLJ	10	46	10G
78	22	6	L	15-08-1994	52/79/80	MLJ	10	76	10G
79	32	6	R	20-01-1992	811	BBB	3	58	14B
80	32	6	R	25-06-1992	811	BBB	3	55	14B
81	32	6	L	15-08-1994	793	NMK	9	34	9J
82	46	6	R	25-06-1992	291	OMC	9	103	9H
83	46	6	R	31-08-1992	292	OMC	9	99	9H
84	20	5	L	09-03-1992	562	PNC	8	2	10G
85	20	5	L	27-04-1992	293	CCC	10	1	9C
86	20	5	L	25-06-1992	811	BBB	10	1	14B
87	20	5	L	06-01-1993	793	AAA	7	0	12A
88	113	5	L	09-03-1992	692	JFH	7	62	10I
89	113	5	L	19-10-1992	811	BBB	10	1	14B
90	113	5	L	14-02-1994	631	OOL	9	24	9C
Occasional carriers									
91	11	4	L	31-08-1992	691	PPB	10	106	9J
92	11	4	R	01-11-1993	331	QCC	7	44	8D
93	11	4	L	15-08-1994	361	QCC	7	65	8D
94	33	4	R	20-01-1992	191	AAA	7	84	12A
95	33	4	L	09-08-1993	793	AAA	7	0	12A
96	33	4	L	15-08-1994	231	CCC	11	23	9C
97	76	4	L	24-02-1992	232	CCC	10	86	9C
98	76	4	L	19-10-1992	191	BBB	10	1	14B
99	76	4	R	15-08-1994	641	OOL	8	7	9C
100	80	4	R	31-08-1992	232	CCC	11	43	9C
101	80	4	L	06-01-1993	692	RNM	8	10	15I
102	80	4	L	14-02-1994	811	BBB	5	12	14B
103	121	4	L	19-10-1992	793	SMH	7	52	10F
104	121	4	L	14-02-1994	811	BBB	5	2	14B
105	121	4	R	14-02-1994	543	PNC	8	2	10G
106	132	4	R	06-01-1993	812	BBB	6	75	14B
107	132	4	L	09-08-1993	811	BBB	6	74	14B
108	132	4	L	14-02-1994	793	PNC	8	2	10G
109	12	3	R	31-08-1992	691	LQN	7	24	—
110	12	3	R	06-01-1993	632	OOL	9	17	9C
111	12	3	L	18-04-1993	811	BBB	5	17	14B
112	15	3	R	27-04-1992	691	BBB	9	0	14B
113	15	3	R	19-10-1992	793	TRO	—	1	—
114	15	3	L	01-11-1993	691	BBB	9	0	14B
115	21	3	R	10-02-1992	231	USC	7	19	17L
116	21	3	R	09-03-1992	232	CCC	10	62	9C
117	21	3	R	18-04-1993	811	BBB	6	84	14B
118	31	3	L	09-03-1992	232	CCC	11	63	9C
119	31	3	L	01-11-1993	311	JFH	10	22	10I
120	31	3	L	15-08-1994	362	JFH	10	8	10I

Note: The carriage index is the number of *S. aureus*-positive cultures in 10 attempts to isolate this species from nasal swabs. Either the left (L) or right (R) nostril was analysed; when both nostrils were examined on a single day, the nostril indication is given in **bold**. Phage types are given in an abbreviated form according to standards applied within the Statens Seruminstitut.

fied. Protein A and coagulase gene RFLP analyses identified nine and 15 types, respectively. However, the overall resolution can be greatly enhanced by combining the datasets (Table 3). As RAPD and coagulase typing results were quite similar, their combination identified a single additional type only. In contrast, the combination of RAPD with protein A types or the combination of protein A and coagulase types recognised 38 and 35 types, respectively (Table 3). This is mainly due to the variability of protein A genes in *S. aureus* strains of otherwise constant genotypic characteristics. It would seem that the protein A gene evolves at a different speed from the coagulase gene or the overall genetic composition as measured by RAPD. For

instance, strains of RAPD type BBB could harbour protein A genes varying in repeat number from three to five to 10 units; RAPD genotypes CCC, FFC, GGG, JIH and JFH displayed a similar protein A gene polymorphism.

When the protein A and coagulase gene variability were plotted against the carriage rate, there was no clear relationship between repeat unit number and colonisation index (Fig. 5). Thus, it can be concluded that repeat length variation and variation at the protein level are not strongly involved in colonisation efficacy. The IgG binding assays, relating protein A content to that of the high-producer *S. aureus* Cowan I, revealed

Fig. 1. Survey of the DNA banding patterns obtained by RAPD analysis of *S. aureus* strains collected from persistent, intermittent and incidental nasal carriers. The primer used was the enterobacterial repetitive intergenic consensus sequence ERIC2. Numbering above the lanes corresponds to that given in Table 1 and identifies the carriers as well; in all six panels, mol. wt markers are included in the right and left lanes. The most intensely staining band identifies an 800-bp DNA fragment; all the other fragments differ by an additional 100-bp fragment. Banding patterns have been interpreted by visual inspection; the corresponding codes are presented in Table 1.

a random distribution of protein A activity when compared to the carrier index (Fig. 5b). This confirms the lack of similarity between gene polymorphism and the same carrier state. Moreover, plotting protein A repeat variability against the relative amount of IgG binding revealed that there was no linear relationship between the two characteristics. A constant number of repeats was accompanied by largely differing IgG-binding coefficients.

Discussion

The human nasal cavity and especially the anterior nares provide an excellent ecological niche for *S. aureus*. Although varying carriage rates have been documented it is generally assumed that a substantial proportion of the human population harbours *S. aureus* at this particular site. Although large studies were performed in the 1960s on the epidemiological behaviour of *S. aureus* [18, 19], there is still little knowledge about the basic determinants of successful long-term colonisation by *S. aureus* and its population dynamics. The human host has shown genetic predis-

position to *S. aureus* carriage in some cases [20]. For instance, race or HLA type may influence this [21]. Numerous bacterial components have been implicated in the initial adherence process that leads to successful colonisation of the nasal epithelium, e.g., specific bacterial receptors for surface proteins, including those for collagen [4], fibronectin [5] and fibrinogen [22]. Several of the corresponding genes have been identified, cloned and subjected to mutagenesis. The latter studies indicate a strongly multifactorial nature in adhesion, colonisation and, probably, infection pathways. Bacterial cell-wall components such as the (lipo)teichoic acids are said to be of critical importance in establishing colonisation [23], but, at present, no mechanisms that are involved in persistent or intermittent interaction between host and bacterium have been elucidated.

Table 1 indicates the genetic diversity encountered among *S. aureus* strains isolated from the human nose. Variation can be observed throughout the entire genome by RAPD, but also more localised, large differences in, e.g., repeat numbers, can be seen. In all cases except one, a single strain was encountered,

Table 2. Comparison of carriage rate and the different number of *S. aureus* phage types and RAPD genotypes detected

Individual code	Carriage index	Number of strains	Phage types		RAPD types		Number of phage types/100 strains	Number of RAPD types/100 strains
			n	%	n	%		
A	10	6	4	66	2	33		
B	10	6	3	50	1	16		
C	10	6	3	50	1	16		
D	10	6	1	16	1	16		
E	10	6	3	50	1	16		
F	10	6	2	33	1	16		
A	9	6	3	50	1	16		
B	9	6	4	66	4	66		
C	9	3	1	33	1	33		
D	9	2	2	100	1	50		
E	9	4	3	75	3	75		
F	9	3	3	100	2	66		
							Persistent carriers	
							57	35
A	8	4	4	100	2	50		
A	7	4	3	75	1	25		
A	6	4	4	100	2	50		
B	6	4	4	100	3	75		
C	6	2	2	100	1	50		
D	6	3	2	66	2	66		
E	6	2	2	100	1	50		
							Intermittent carriers	
							92	52
A	5	4	4	100	4	100		
B	5	3	3	100	3	100		
A	4	3	3	100	2	66		
B	4	3	3	100	2	66		
C	4	3	3	100	3	100		
D	4	3	3	100	3	100		
E	4	3	3	100	3	100		
F	4	3	3	100	2	66		
A	3	3	3	100	3	100		
B	3	3	3	100	2	66		
C	3	3	3	100	3	100		
D	3	3	3	100	2	66		
							Incidental carriers	
							100	85

Fig. 2. Plot of RAPD consensus types against individuals with different carriage rates. RAPD type BBB occurs independently of the carriage rate in 17 out of 31 individuals.

Fig. 3. RFLPs generated by restriction endonuclease analysis of coagulase gene amplicons. Lane numbering corresponds to strain numbers given in Table 1. Mol.wt markers are shown on the left.

Fig. 4. Survey of protein A gene typing data obtained for the *S. aureus* strains from individuals demonstrating various carriage indices. Strains are ordered as in Table 1.

Table 3. Comparison of types identified by RAPD, protein A gene typing and coagulase gene typing

RAPD type	Protein A gene type	Coagulase gene type
AAA	7	12A
BBB	3, 5–10	14B
CCC	7, 10	9C
DDD	4	9C
EEE	7, 9	8D, 10G
FFC	6, 7	8D
GGF	-	5E
GGG	9, 10	10F
IHC	7	9H
JFH	7, 8, 9	15I
KJI	9	9C
LKC	8	11N
MLJ	10	10G
NMK	9	9J
OMC	9	9H
PNC	8	10G
JFH	7, 10	10I
OOL	9	9C
PPB	10	9J
QCC	7	8D
RNM	8	15I
SMH	7	10T
LQN	7	-
USC	7	17C

defining single isolate long-term persistency. As most of the strains were genetically distinct, it is not possible to identify any factors in the nose that favour selection of *S. aureus*. It is noteworthy that in the low

index carriers putative persistence can sometimes be observed. In individuals 31, 15, 132, 33 and 11, an identical strain was isolated twice, sometimes years apart (types JFH, BBB, BBB, AAA and QCC, respectively). This means that in 50% of the low-score individuals, the lack of persistence as defined by bacterial cultivation may be due to low or variable numbers of bacteria in the nares. On the other hand, it could also be that although multiple types are present, only a single strain is cultured. Table 1 shows three instances of single-day sampling of both nostrils. In one of these, the host was colonised by different strains in different nostrils.

Several bacterial surface proteins are thought to play an important role in the processes of adherence, colonisation and infection [6], e.g., the immunoglobulin G binding protein A [24]. It has been suggested that IgG binding interferes with bacterial opsonisation [25–27], a feature that would enable evasion of the host immune response by the bacteria. However, the reports on the relevance of protein A expression with regard to virulence or pathogenicity have been contradictory. Where initial data indicated that differences in the level of protein A production resulted in changes in bacterial virulence [28], later studies failed to confirm this [29]. More recently it has been suggested that variability in the protein A

Fig. 5. Schematic overview of repeat unit numbers in the coagulase gene (a), the protein A gene (c) and the relative amount of protein A (b), plotted against the different individuals displaying different carriage rates for *S. aureus*.

gene repeat region may be correlated with the epidemic behaviour of *S. aureus* strains [16]. The longer the repeat region, the more 'epidemic' a strain would be. This could be due to extended exposure of the immunoglobulin-binding domain and subsequent improvements in immune evasion. In the present study, a similar correlation between the length in protein A gene repeats and persistence of colonisation was shown not to exist. Thus, the hypothesis that larger numbers of repeats may result in improved evasion from the immune system, thereby rendering long-term colonisation more likely, may be incorrect. This is further supported by the fact that there appears to be no linear relationship between the number of repeats and the amount of protein A exposed on the bacterial cell wall. The fact that there is this lack of relationship indicates that it is impossible to predict the biological behaviour imposed by protein A from the number of repetitive moieties present in the encoding gene. In any case, this particular feature does not seem to play a major role in differentiating long-term from short-term nasal carriers of *S. aureus*; or it could be that a large proportion of the strains studied produced extracellular protein A. As the latter is a feature of methicillin-resistant *S. aureus* mainly [30], this is unlikely.

Although conflicting data have been presented in recent literature it is currently believed, based on site directed allele replacement studies [31], that coagulase is probably not a major virulence determinant of *S. aureus*. The repetitive region within the gene is no absolute requirement for prothrombin binding and plasma clotting at the protein level. However, this region may play a role in binding to the bacterial surface. Regarding the structural homologies with similar repeats in protein A and protein G genes, these may be responsible for the strict adherence of a portion of the largely extracellular protein to the cell wall of *S. aureus* [32]. As such, the quantity of this membrane-bound fraction could depend on repeat unit length. This again could have a direct effect on the way in which the staphylococcal strain interacts with its environment. For this reason, we studied whether or not there was a relationship between persistence of colonisation and the composition of the coagulase gene. As was the case for the protein A gene variability, no such correlation appeared to exist. On the other hand, the data confirmed the usefulness of coagulase gene variation for typing of *S. aureus* strains [17, 33].

It has been demonstrated recently that the incidence of isolation of *S. aureus* phage type 95 has increased to 20% of the total number of *S. aureus* strains from human clinical material in the Danish population [34]. In the present study, it was shown that this particular type was present in over 50% of the subjects. The putative clonal nature of this strain, which is resistant to penicillin only, was confirmed by the data obtained

by RAPD studies. All the isolates of phage type 95 belonged to RAPD type BBB, indicating true genetic homogeneity, irrespective of the fact that within the protein A genes of these strains variable repeat numbers can be determined.

In summary, the results of phage typing and RAPD analysis in this study could not demonstrate any common genetic or phenetic elements in defined groups of nasal *S. aureus* carriers. Genetic variation in two genes encoding proteins exposed outside the staphylococcal cell does not seem to contribute to variation in persistence; this was confirmed by the phenotypic analysis of the gene product of one of these two.

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