Nasal carriage of staphylococci in an Antarctic community

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Healthy carriers of *Staphylococcus aureus* can be harmful to themselves and to the community (Williams, 1963; Ayliffe *et al.*, 1977; Bengtsson, Hambracus & Laurell, 1979). As authors are far from unanimous regarding the reasons or factors which upset the symbiosis of this potential pathogen with its host, the carriage state is of central importance. Most intensive investigations of nasal carriage have been carried out in hospitals where the large number of casual contacts including doctors, nurses, ancillary staff and visitors, along with the rapid turnover of patients with an inherent reservoir of potentially virulent organisms, have made analysis difficult.

Isolation of small groups of men in submarines, space vehicle analogues and space craft has enabled studies to be made of the transfer of staphylococci among contacts (Watkins, 1970; Gordon, 1970; Taylor *et al.*, 1977). In all the space isolation studies, however, there was a maximum of only three subjects, while in the submarine and spacecraft analogue experiments, although larger numbers participated, the longest period of isolation was 60 days: sampling was often infrequent and swabs were not taken from all of the confined men.

Antarctic bases (fig. 1) provide sites where isolation is uninterrupted from 7–13 months. Expedition members are all young healthy male volunteers who cooperate well with medical investigations. Five bacteriological studies have been undertaken in the Antarctic by Australian, Russian and British workers. McLean (1919) noted that 'Staphylococci (white in culture)' persisted in the noses of several expedition members throughout the 9-month period, but 'Staphylococcus pyogenes aureus' although present during the first 3 months was not isolated during the last 6. Between 1948 and 1951, Sladen (1953), the British expedition medical officer, showed that isolation and the Antarctic had no inhibitory effect on the normal commensals: throughout two years coagulase-positive staphylococci were

isolated from some of the expedition members' noses but he noted that the numbers of intermittent and occasional carriers of coagulase-positive staphylococci decreased. Phage-typing revealed no transfer of organisms even when subjects shared a tent.

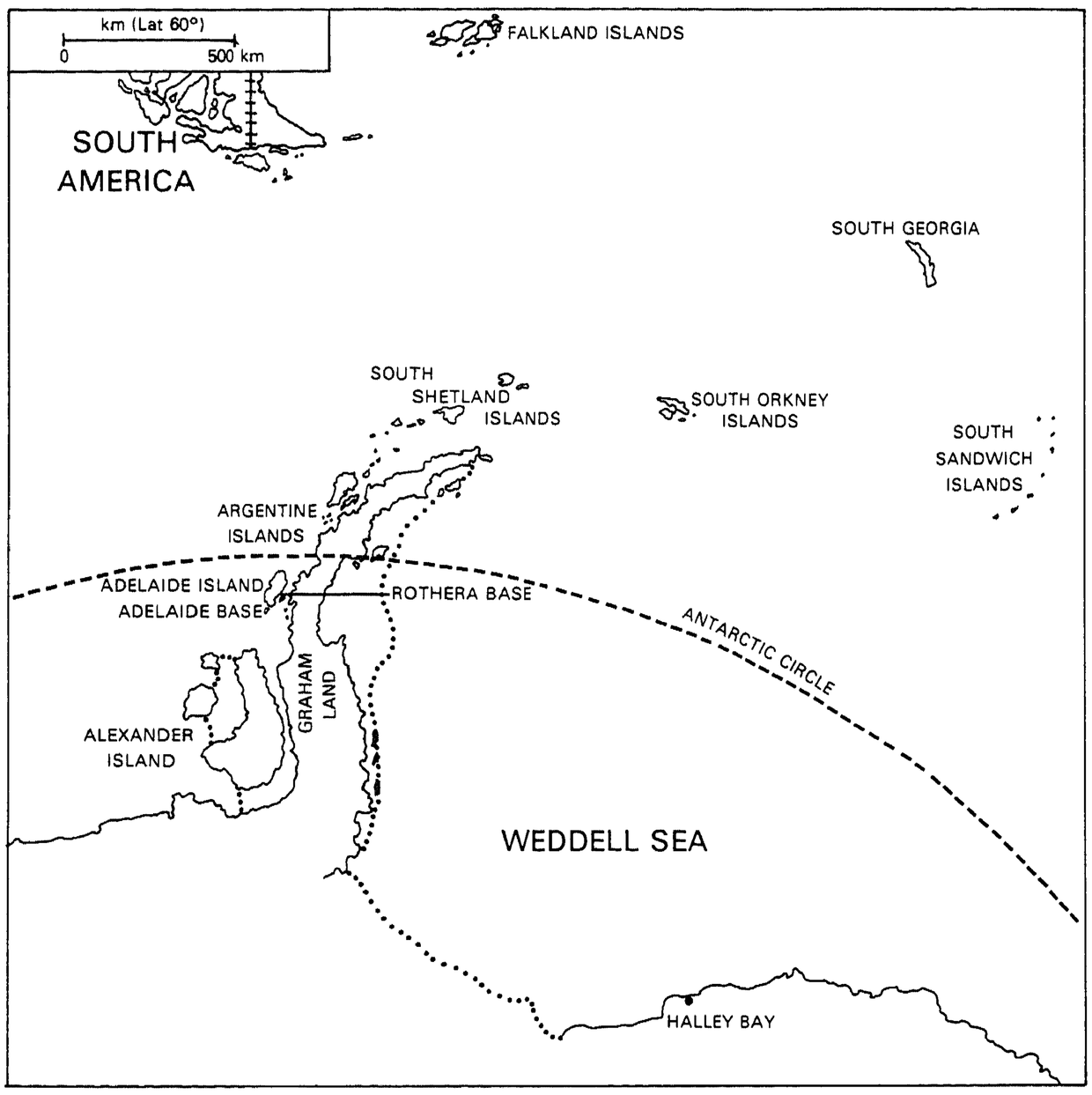

Fig. 1 British bases in the Antarctic.

Cameron (1968) studied the staphylococcal nasal flora of Australians at Mawson Station at monthly intervals for ten months. He found that the rate of persistent nasal carriage of coagulase-positive staphylococci was stable at 50 per cent; a rate he considered comparable for normal populations in temperate zones. Only occasional carriers were found in the Mawson group and phage-typing indicated that their strains were closely related to those

isolated from their sledging partners. The carriage rate of coagulase-negative staphylococci in the Mawson party quickly rose to 100 per cent and remained at that level throughout isolation.

Williams (1969) found only 3 nasal carriers of coagulase-positive staphylococci when the 13 base members on Stonington Island were swabbed at monthly intervals for a year. Even they gave a low yield of positive isolates (6 instances out of 27 samples). Phage typing revealed that each carried a distinct strain, and no transfers of organisms to non-carriers took place. During the 12th and 13th Soviet Antarctic Expedition (1967–8), Tashpulatov and Petrosov, (1973) found that microorganisms isolated from the nasal mucosa showed a gradual decline in numbers during the course of the winter, falling to one-quarter of their pre-Antarctic levels. Haemolysis on blood agar plates was considered to be the index of pathogenicity and no organisms were fully identified.

In the five investigations of Antarctic personnel mentioned above, swabbing of the anterior nares was completed at most once per month and often there was an even longer period between swabs.

The Adelaide Island Study

A study of the carrier state of the British Antarctic Survey (B.A.S.) expedition members on Adelaide Island and the influence of the environment of the base was carried out between 1975 and 1978. The study included weekly nasal swabbing, swabbing of pyogenic lesions, air sampling with settle plates in the base buildings and sweep plates of sleeping bags and tents used on base and during sledging expeditions. Since all the base members came into contact with the local fauna directly or indirectly—seals, penguins, gulls, petrels and the sledge dogs, attempts were made to isolate *S. aureus* from the anterior nares of the 45 huskies kept on Adelaide Island and from the penguins which passed through the base area. Since claims have been made that dogs harbour and transmit staphylococcal strains pathogenic for man (FAO/WHO, 1967; Live, 1972; Meeks, 1978) they may form a potential source of staphylococci where humans are often in close contact with huskies.

Methods and Materials

The subjects

Of thirteen men isolated on Adelaide Island four wintered at an advance base, Rothera, on the east side of the island, while the remainder lived on its

southern tip. Several field trips involved an interchange of personnel between these bases when Adelaide Island was isolated. There was one partial break in isolation when an Argentinian plane collected three Adelaide base members during an attempted rescue of a lost climbing party from another British base. They flew to an American base, and stayed there for nearly three weeks. On return of two British members (the third stayed at Palmer following the development of a cerebral tumour) the four Argentinian aircrew remained at Adelaide for a week. These episodes were monitored with nasal swabs taken before, during and at the end of the visit.

Collection techniques

Air sampling in the living quarters, workshops, field camps and in the open air close to the huts was carried out using settle plates, the only method possible in the circumstances. Open 9 cm Petri dishes of Columbia agar (CM 331, Oxoid) were exposed for 2 h at appropriate sites. Sampling was carried out twice in each season during isolation. The indoor samples were collected in duplicate, once during maximum activity and once during minimum activity. Eight plates were also exposed in four outside areas at each sampling period. Each exposed dish was placed on top of 0.5 m high stool to avoid freezing at ground level.

Sweep plates (Williams *et al.*, 1966, Speers *et al.*, 1969) of the base members' blankets and the mouths of sleeping bags were taken. After field trips the inner fabric of the tents was also sampled. Ten sweeps produced an optimum sample and this was carried out twice in each season during isolation.

Nasal swabs were collected in duplicate from all huskies twice during the isolation period on weeks 2 and 23. Ten penguins were swabbed from beaks, breathing tubes and underwing feathers, (where they rubbed their beaks), twice in the spring, on weeks 28 and 32. Techniques of processing were the same as those used for the human nasal swabs.

The base members

Both anterior nares and septa were sampled with a dry sterile plain cotton-wool tipped swab (Exogen) using a firm rotary motion. As all the subjects wore beards and moustaches at some time during their Antarctic sojourn, care was taken to avoid those areas when swabbing. Incidents of sepsis among the men were recorded and lesions swabbed.

Laboratory procedures

Columbia agar (CH 331, Oxoid) was used for all isolations unless otherwise stated. Swabs taken from the men, dogs and penguins were plated and

incubated with the settle and sweep plates at 36°C for 24 h. Those taken in the field were immediately inoculated on to pre-warmed, agar slopes in 5 ml bijou bottles. They were incubated in the subject's pocket next to his skin during the day and at night in a small pouch in his sleeping bag. This prevented the media from denaturing on freezing and allowed the bacteria to incubate at between 28 and 36°C for up to 10 days while the men were sledging. Once the party returned to base the mixed growth was plated out and a minimum of 5 representative colonies of each morphological type were selected and cultured for 18 h.

Second passage colonies from each of the primary cultures, chosen on morphology and staining characteristics were tested by the slide coagulase method. The coagulase-negative Gram-positive cocci and Gram-negative 'non-swarming' and 'swarming' bacilli were also noted. Representative isolates were cultured on Dorset egg medium (without crystal violet, PM5p, Oxoid,) or on agar slopes in bijou bottles and incubated at 36°C for 18 h, and then stored at between 2 and 7°C later to be transported to the Department of Bacteriology, University of Aberdeen.

Cultures were revived by incubating them at 37°C for 48 h in 3 ml of tryptose phosphate blood broth. A loopful of the incubated broth was then cultured on blood agar. Slide coagulase tests were repeated on these isolates and equivocal results were checked by the tube method.

Phage typing was carried out on all coagulase-positive pure isolates using the 24 phages of the International set.

A selection of strains was also tested with 4 canine phages 93, 06, 40 and 58 supplied by the Staphylococcus Reference Laboratory Colindale while those from penguins were sent to Dr J. T. Patterson at the Agriculture and Food Science Centre, Queen's University of Belfast, for biotyping and testing against a set of poultry phages as described by Gibbs, Patterson & Thompson (1978).

All coagulase-positive strains were also tested for sensitivity to antibiotics by the agar diffusion method (Ericsson & Sherris, 1971).

Species differentiation of Gram-negative swarming bacilli was made using the API 20E system (API System SA, Montalieu-Vercieu, France), and the Dienes (mutual inhibition) test was performed.

RESULTS

Staphylocci in the environment

Of 348 plates exposed, 317 plates (91 per cent) did not yield colonies of *S. aureus*. Twenty-four yielded one colony while only 7 yielded 2 colonies. No plates were found to have more than 2 colonies.

The mean count over the whole isolation period was 7.8 col./m^2 h. During the 15th week of isolation, the coldest week at the base, *S. aureus* was not isolated. Only 7 *S. aureus* strains were recovered during the 6 'low activity' periods. All 5 separate phage-types were found (table I). Only 4 of the positive plates yielding two different types, while 29 had one type. Of the plates each with 2 *S. aureus* colonies 3 carried the same type and 4 carried different types.

TABLE I

The number of colonies of each phage type of *S. aureus* grown from settle plates exposed for 2 h on each week of sampling at Adelaide Base

Week No.	NT/NT	29/+	94/96	47/53/88/+	NT/52A/80	Total
0	3	3	2	1	—	9
5	—	2	2	—	—	4
11	1	5	2	—	—	8
15	—	—	—	—	—	—
24	—	2	2	—	2	6
26	2	5	2	—	2	11
TOTAL	6	17	10	1	4	38

No coagulase-positive staphylococci were isolated from the 96 plates exposed out-of-doors but one coagulase-negative staphylococcus colony and many yeasts were isolated from their frozen surfaces.

At one field camp during the tractor traverse of Adelaide Island on week 17, 4 plates were exposed for 2 h inside two tents. As with the outdoor samples, the agar froze within 30 min of exposure to the ambient temperature at sleeping bag level. On return to base only moulds were grown.

Fifty-four sweep plates were made from the mouths of the base members' sleeping bags and blankets. Table II shows the phage types of the coagulase-positive staphylococci found. Only 3 phage types of staphylococci were isolated from fomites and were not found in base members' noses. Four other types were found in human carriers.

Nine tents, returned to base following field trips yielded only 3 isolates of *S. aureus* from sweep plates.

TABLE II

The phage types of the *S. aureus* grown on sweep plates made from each base member's sleeping bag

Week no.	Subjects 1	2	3	4	5	6	7	8	9
5	NT/NT	–	–	29/+	47/53/88/+	77 29/+	94/96	–	NT/NT
10	–	–	–	29/+	NT/NT	29/+	94/96	94/96	–
15	29/+	–	–	29/+ NT/NT	–	29/+	29/+	–	6/53/77/84/85/+
20	–	–	–	29/+	–	–	–	NT/NT	–
25	29/+	–	29/+	29/+	–	29/+	–	–	–
30	29/+ 29/52/79/80/42E/75/84/+	94/96	–	29/+	NT/NT	29/+	94/96	–	–

One hundred and eighty nasal swabs were cultured from the 45 huskies. Coagulase-positive staphylococci were isolated from 46 of the swabs. None of these staphylococci were typable with the human International set of phages at RTD or 100×RTD but 10 were typable with the four experimental canine phages offered by Colindale: 8 of the strains were typed 06/40/58 and seven of the dogs carried the same strain when tested 21 weeks later.

More than half the canine strains tested were resistant to penicillin. From 20 penguin swabs 6 yielded coagulase-negative staphylococci and 2 coagulase-positive staphylococci. These were further investigated but were neither typical of human or recognized avian strains.

The base members

Adelaide base. Although coagulase-positive staphylococci were isolated from each of the 9 men during isolation, only 4 were persistent carriers (table III). One man (subject 1) became a constant carrier for the last 3 months. This contrasts with his first 5 months when only sporadic isolations were made. Swabs from the 4 occasional carriers (subjects 2, 3, 5 and 9) also yielded only 3–6 scattered isolations of the organism throughout the period they were on base. Some men resist colonization by *S. aureus* but may act as temporary hosts.

When the phage type and antibiotic resistance patterns of the nasal staphylococci from occasional carriers were considered, no obvious source of these organisms was found. Some phage types, such as type 94/96, resistant to penicillin and the type 29/+ also resistant to penicillin, but also occasionally resistant to streptomycin and lincomycin, were present in the anterior nares of persistent carriers. They were also isolated from some of the settle plates used to sample the environment as early as week 0. *S. aureus* type 47/53/88/+, consistently penicillin-resistant although not grown from a base member's nose before week 1, was isolated as a single colony during week 0 from the bunkroom corridor. There was still doubt as to the source of the staphylococci NT/52A/80, NT/NT (weak 52A), and NT/NT (V.V. weak 95) all fully sensitive to antibiotics. It was possible that these could have been acquired on field trips to Rothera. The effect, therefore, of such field trips between the two bases isolated on Adelaide Island was studied.

Rothera base. As with Adelaide base members, coagulase-positive staphylococci were isolated from each of the Rothera personnel at some time during the 33 weeks of isolation (table IV). Several exchanges of nasal coagulase-positive staphylococci appeared to have taken place. The NT/52A/80 staphylococcus in particular was found initially at Rothera in

TABLE III

Phage types of *S. aureus* from nasal swabs of Adelaide base members

Week of isolation	Subjects 1	2	3	4	5	6	7	8	9
Before	...	–	...	–	...	c	a	b	–
0	–	–	–	b	–	c	a	a	–
1	–	–	–	b	d	c	a	a	–
2	–	–	–	b	d	c	a	a	–
3	–	–	–	b	–	c	a	a	–
4	–	–	–	b	d	b	a	a	–
5	d	–	d	a	d	b	a	a	–
6	–	–	–	b	–	b	a	a	–
7	–	–	–	b	–	b	a	a	–
8	b	–	–	b	–	b	a	a	–
9	+	–	–	b	d	c	a	a	–
10	d	–	–	b	d	c	a	a	–
11	–	–	–	b	–	b	a	a	–
12	–	–	–	b	–	b	a	a	–
13	–	–	–	b	+	b	a	a	–
14	–	–	–	b	–	b	a	–	–
15	b	–	–	b	–	b	a	a	–
16	–	–	–	b	–	b	a	a	–
17	–	–	e	b	–	b	a	–	–
18	...	...	...	...	...	...	...	...	...
19	b	–	–	b	–	b	a	–	–
20	–	–	–	b	–	b	a	–	–
21	...	...	...	...	...	...	...	...	...
22	...	...	...	...	...	...	...	...	...
23	b	b	–	b	–	b	a	b	b
24	b	–	–	b	–	b	a	a	–
25	b	–	–	b	–	b	a	a	–
26	b	–	–	b	–	b	a	b	–
27	e	–	e	b	–	b	a	a	e
28	b	–	b	b	–	b	a	ba	a
29	b	a	–	b	–	b	a	...	–
30	b	–	–	b	–	b	a	...	–
31	b	–	–	b	–	b	a	...	–
32	b	g	b	b	–	b	a	...	–
33	b	–	b	b	–	b	a	...	–
after	–	b	–	–	–	b	a	b	–

S. aureus phage types
a 94/96
b 29/ +
c 77
d 47/53/88/ +
e NT/52A/80
g NT/NT (95)

– *S. aureus* not grown
+ *S. aureus* isolated but not phage typed
... no sample available

subject 10, then on two occasions in Adelaide personnel. It was isolated first in subject 3 during the tractor train, and then 10 weeks later in subjects 1, 3 and 9. Its origins before week 10 at Rothera remain obscure. Rothera base members probably acquired two organisms from Adelaide base personnel. Both were short-lived—phage type 94/H96 in subject 13 on weeks 9 and 11, and the type 29/+ in subject 10 on weeks 23, 24 and 25. The two men were staying at Adelaide base when these staphylococci were isolated. Although not grown from Adelaide base members, nasal staphylococcus NT/29/52/51A/80/+ was found in subject 10 while he was visiting Adelaide on week 26. Following his return to Rothera on week 29 this phage type of coagulase-positive staphylococcus was isolated from the other three Rothera base members.

Argentinian flying party. On arrival at Adelaide none of the Argentinians' nasal swabs grew coagulase-positive staphylococci. Those taken at the end of the first week on base showed that one of the aircrew had acquired staphylococcus type 94/96 which was resistant to penicillin and lincomycin. The other three men remained non-carriers. The same results were found before they left early in week 29. During their visit type 94/96 had been acquired by two of the expedition occasional carriers for one week each. It was noted that the two Adelaide men brought back from the American base did not change their carriage patterns. Subject 4 remained a persistent carrier of type 29/+ while subject 5 continued to be a non-carrier.

Staphylococcal sepsis in base members. Table V summarizes the findings from swabs of septic lesions. Nearly half of the Adelaide Island expedition members suffered from staphylococcal sepsis at some time in the 33 weeks. All the Adelaide base personnel who were persistent carriers and the one intermittent carrier of nasal *S. aureus* had lesions. The one Rothera man with staphylococcal lesions was also a persistent carrier of a nasal staphylococcus. Because the men lived in such close contact, transfers of lesion-producing organisms between them might have been expected (Kay, 1962). This only appeared to occur in one subject. The other lesions from which coagulase-positive staphylococci were isolated, yielded either the same phage type as the individual carried in his nose or a phage type which had not been isolated on base before.

The episodes listed in table V may be likened to the minor trauma experienced by engineering workers (Williams & Miles, 1949) or miners (Atkins & Marks, 1952; Roux, 1958) where staphylococcal infections were acquired through employment. Styes experienced by subjects 8 and 10 were probably initiated by the frequent necessity to remove the build-up of rime ice from eyebrows, eyelashes and beards when working and travelling in

TABLE IV

Phage types of *S. aureus* from nasal swabs of Rothera base members

Week of isolation	Subjects 10	11	12	13
Before	k	b	...	–
0	...	...	...	...
1	...	...	...	...
2	...	...	...	...
3	k	b	–	–
4	...	...	...	...
5	...	...	...	...
6	k	b	–	–
7	k	b	–	–
8	...	b	...	...
9	...	b	...	a
10	e	b	...	–
11	...	b	...	a
12	e	b	h	–
13	e	b	–	–
14	...	...	...	...
15	e	+	–	–
16	...	...	...	...
17	k	–	–	–
18	...	...	...	...
19	...	...	...	...
20	+	–	–	–
21	...	...	...	...
22	...	...	...	...
23	b	+	–	–
24	b	+	–	–
25	b	–	–	–
26	p	+	–	–
27	h	b	–	–
28	...	...	...	...
29	–	–	p	p
30	...	...	...	...
31	–	p	p	–
32	...	...	...	...
33	–	p	p	–
after	b	b	–	–

S. aureus phage types
a 94/96
b 29/ +
e NT/52A/80
h NT/NT (52A)
k NT/NT (80)
p NT/29/52A/80/ +

– *S. aureus* not grown
+ *S. aureus* isolated but not phage typed
... no sample available

TABLE V

Phage-types of staphylococci isolated from base members' septic lesions and concurrent, nasal swabs

Subject	Week isolated	Lesion	Organism isolated	Nasal carriage
1	4	Wound infection following dog bite	NT/52A/79/+, fully sensitive	47/53/88
	30	Furuncle on back of neck	29/+, resistant to penicillin/lincomycin	29/+
2	–	–	–	–
3	14	Pustule on arm	No staph. but diphtheroids	–
4	1	Furuncle on neck	29/+, resistant to penicillin/lincomycin	29/+
	19	Paronychia finger	29/+, resistant to penicillin/lincomycin	29/+
5	–	–	–	–
6	28	Paronychia toe	29/+, resistant to penicillin/lincomycin	29/+
	33	Conjunctivitis	NT/NT, fully sensitive	29/+
7	28	Furuncle chest	94/96, resistant to penicillin/streptomycin/lincomycin	94/96
8	11	Stye	94/96, resistant to penicillin/streptomycin	94/96
9	29	Furuncle on leg	No staph. ? sterile	–
10	10	Stye	29/+, resistant to penicillin	NT/52A/80
	31	Stye	NT/29/52/52A/80/+, resistant to penicillin/streptomycin	–
11	–	–	–	–
12	–	–	–	–
13	–	–	–	–

extreme cold. This inadvertent plucking of hairs may have predisposed the follicle to infection.

Of 346 nasal swabs examined, 246 yielded coagulase-negative staphylococci and 69 yielded gram-negative organisms of which 21 were 'swarming' *Proteus spp.* while 76 swabs grew neither coagulase-negative staphylococci nor gram-negative organisms. Gram-negative non-swarming organisms were never isolated from the same nasal swab as *Proteus spp.* However, gram-negative organisms and coagulase-negative staphylococci were isolated from the same swab on 49 out of 69 occasions.

One subject appeared to be a constant carrier of a *Proteus spp.* and although only 5 of his isolates survived storage it is likely he carried the same strain of *P. mirabilis*. This assumes that storage conditions did not select out this, the only surviving strain.

IV Discussion and Conclusions

The isolated Antarctic base provided a small closed community in a 'simpler' environment than that found in hospital wards or that of the unconfined general population.

Environmental contamination was low, confirming previous reports. *S. aureus* was not isolated from settle plates out of doors. Indoors, airborne contamination with *S. aureus* appeared to exist at levels between those of a hospital ward (Lidwell *et al.*, 1975) and a domestic household in a temperate climate (Finch, Prince & Hawsksworth, 1978). No previous Antarctic studies have isolated coagulase-positive organisms from the air during the winter period (McLean, 1919; Cameron, 1968). Evidence suggested that sleeping bags were responsible for the transfer of several of the coagulase-positive strains. This contrasts with the Australian study where Cameron (1968) had detected only coagulase-negative staphylococci from his expedition members' sleeping bags, although 50 per cent of the personnel were nasal carriers of *S. aureus*.

Nearly one-third of the sledge dogs at Adelaide were nasal carriers of *S. aureus*. Their human handlers did not become infected by the canine strains during the period of the study. However, after the isolation period of the study had ended, one member was found who was carrying a canine strain in his nose. Any future study would require to take such possible transfers into account. The only two penguin isolates were specific for birds.

When human nasal carriage before leaving for the Antarctic was compared to that observed for up to two years following return to the United Kingdom, few changes appeared to have taken place. Only one

additional carrier was noted among the Adelaide Island personnel. By contrast, during the time when the men were isolated on base, many transfers of *S. aureus* took place. This is contrary to previous findings in the Antarctic. Several transfers of distinct strains of coagulase-positive staphylococci between persistent carriers were noted while the number of non-carriers who became occasional or intermittent carriers was similar to that found in patients admitted to hospitals in more temperate climates (Williams *et al.*, 1962; Ayliffe *et al.*, 1977). The source of these dispersed strains was only revealed because of the frequency of swabbing—once per week.

Most septic lesions were related to incidents of minor trauma. The strains most frequently implicated in the production of these lesions at Adelaide base were type 29/+ and type 94/96 staphylococci. Both were isolated from subjects who were already nasal carriers of these phage types. It was found that nasal acquisition of types 29/+ and 94/96 by occasional carriers occurred in the three weeks around the appearance of a lesion in a persistent carrier. It was not possible to determine whether this was due to increased shedding of the organism from the anterior nares or elsewhere or from the lesion, because occasional carriers also acquired strains on several occasions when there were no septic lesions present in base members.

It is possible that the character of the coagulase-positive staphylococci studied during the period 1975–8 was different to that found in the previous studies reported in 1953, 1968 and 1969. Thus, in the 1968 study only one-third of strains isolated were resistant to penicillin while in the 1969 study all were penicillin-sensitive. The majority of strains isolated from the Adelaide Island personnel in the present study were penicillin-resistant. In 1973 Altemeier and his colleagues suggested that there might be a cyclical variation in the antibiotic sensitivity of *S. aureus*. It may be that such a variation was taking place during the Adelaide Island study and that this manifested itself as the ability of the *S. aureus* to transfer to and colonize others.

As many or more transfers of pyogenic staphylococci occurred in the present study as are reported in other studies of closed or semi-closed communities. The idea that intercurrent infection or colonization by *S. aureus* is less likely to be acquired by fit young men living in a supposedly bacteriologically sterile external environment in the Antarctic, is not sustained.

SUMMARY

Contrary to previous reports both coagulase-negative and -positive staphylococci flourished in the anterior nares throughout Antarctic winter

isolation. Several transfers of *S. aureus* between personnel were identified by phage type and antibiotic sensitivity. The main agent for transfer appeared to be the sleeping bags used on base and on field trips. Staphylococci were isolated from one-third of the huskies tested and it is possible that they could be a source of human infection.

Minor septic lesions were related to the strains of *S. aureus* present in base members' anterior nares.

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