Variation in the Prevalence of Antibiotic Resistance of *Staphylococcus aureus* from Human Skin and Nares

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SUMMARY

Patients whose skins are colonized with *Staphylococcus aureus* resistant to penicillin and/or tetracycline may carry variants that are sensitive to these antibiotics. The skin of most individuals yields the fully resistant type as the predominant flora and the nose harbours the sensitive version. This probably represents plasmid loss *in vivo*. The plasmid-positive cells were not more resistant to desiccation or more deeply pigmented than plasmid-negative cells. The explanation for such distributions is unknown.

INTRODUCTION

Few of the factors which affect growth of micro-organisms on the skin are well understood, although increased relative humidity and temperature cause an increase in the total flora and the application of antibiotics causes changes in the flora. The factors which permit colonization by *Staphylococcus aureus* of the skin of patients with eczema and psoriasis or prevent colonization in a normal host are not known, though these might include requirement for specific nutrients or the presence of antibiotic-producing strains amongst the normal flora.

Microbial variants which differ in some easily measured property have been studied extensively *in vitro*, but have been examined less often in their natural habitat. Two resistance markers are well characterized in *S. aureus*; these are penicillinase production and tetracycline resistance. The genes controlling these resistances are located on separate plasmids (Lacey, 1975). The penicillinase plasmid carries information for heavy metal ion resistance and some other properties; the other plasmid governs only tetracycline resistance in the strains so far examined. The genes for pigment production are not carried by the above plasmids but are linked to resistance to desiccation and to linoleic acid, a component of human sebum (Grinsted & Lacey, 1973).

In 1972, Noble reported strains of *S. aureus* obtained from patients with skin disease in which antibiotic resistance appeared to be lost *in vivo*. This paper reports further studies on unstable antibiotic resistance in staphylococci in their natural habitat.

METHODS

Patients were swabbed in the nose, on the chest, perineum, right and left forearms, and right and left thighs using cotton-tipped swabs moistened in nutrient broth. The swabs were inoculated on to blood agar (Oxoid blood agar base containing 7% lysed horse blood) and incubated for 24 h at 37 °C; five plates were used for each swab.

All single colonies of *S. aureus* on selected plates were subcultured and tested for resis-

tance to penicillin and tetracycline on a blood agar medium. The criterion for resistance to an antibiotic was an inhibition zone less than 21 mm diameter surrounding a disc containing 10 units of penicillin, or 18 mm surrounding a disc containing 30 µg tetracycline on Difco Mueller-Hinton medium containing lysed horse blood.

Attempts to induce variants that were antibiotic sensitive or had altered pigmentation were made using Oxoid nutrient broth containing ethidium bromide (1 mg l⁻¹) or dithranol (saturated) incubated at 37 or 43 °C. Dithranol (1,8,9-trihydroxyanthracene) is used extensively in the treatment of psoriasis and has been shown to induce mutants in yeasts (Gillberg, Zetterberg & Swanbeck, 1967). Serial 10-fold dilutions were made and all single colonies from suitable plates were tested as above.

Staphylococci were inoculated on to porcine skin used in burns treatment (Lyoderm, Armour Pharmaceutical Co., Phoenix, Arizona, U.S.A.) to study their survival on drying. The skin was divided into 1 cm squares and saturated with Murphy's synthetic sweat solution (Murphy, 1975). Some squares were also treated with the ether-soluble fraction of human sebum, approximately 0.4 mg sebum being added to each square. Washed staphylococci suspended in Murphy's synthetic sweat solution were inoculated on to the squares which were allowed to dry at room temperature with uncontrolled relative humidity. Survival curves were obtained by vigorously shaking squares of skin bearing staphylococci in buffer pH 8.0 containing 0.5 % (v/v) Tween 80 on a mechanical shaker for 1 min; dilutions were then inoculated on to blood agar and the colonies were counted after 24 h incubation at 37 °C. Duplicate samples were used in each instance.

Representative samples were phage-typed using the internationally agreed set of phages. Isolates were assumed to be of the same origin if the phage lysis pattern was the same.

Pigment production was studied on Oxoid blood agar base medium alone or with the addition of Tween 80 (0.1 %, v/v), squalene (0.1 %, v/v), or glycerol monoacetate (0.1 %, v/v) (Willis & Turner, 1962), or with both Tween 80 and squalene (each 0.1 %, v/v). Bacteria were scraped off the plates in saline and washed by centrifuging. Pigment was extracted in methanol for 30 min at 90 °C and its spectrum was examined. Pure β-carotene (Sigma) was used as a standard.

RESULTS

A systematic search for strains of staphylococci with variations in antibiotic resistance has not been carried out, but in 3 months 10 patients were identified from routine clinical specimens as carrying *S. aureus* strains of more than one sensitivity pattern. These patients were amongst 152 with eczema or psoriasis, 106 of whom yielded *S. aureus* from skin swabs.

Thirty patients were examined at all carriage sites; 16 had staphylococci that were 95 % or more consistent at all sites and are not included in further analyses, except to note that in only three instances were the sensitive varieties more numerous than resistant varieties. Ten patients carried strains varying in their resistance to tetracycline and four to penicillin. The strains and their characteristics are shown in Table 1 in the order of detection. No particular phage type was encountered, though two pairs of patients may have carried the identical strain (7 and 8, 9 and 11).

Table 2 shows the distribution of the resistant and sensitive variants on the body surface. In general, with both penicillin and tetracycline, the skin carried the largest proportion of resistant isolates and the nose the sensitive ones. Isolates from the perineum followed the 'skin' pattern rather than the 'nose' pattern. On 12 occasions the skin flora possessed proportionately more than twice as many resistant cells as the nose; on no occasion did the

Table 1. *Characteristics of patients and organisms in order of detection*

Patient	Sex	Diagnosis	Treatment			Phage type of Staphylococcus	Resistance pattern*	Labile resistance	Pigment difference (see text)
			Dith- ranol	Topical steroid	Antibiotics				
1	M	Atopic eczema	—	+	—	80+	P	P	NIL
2	M	Pustular psoriasis	—	+	—	6/42E/47/54/75/77/84/81	PTNE	T	R/T darker
3	M	Psoriasis	—	+	—	84/85	PTNE	P (and T)	NIL
4	M	Atopic eczema	—	+	—	85	PT	T	R/T darker
5	F	Atopic eczema	—	+	Topical Cn	52/52A/80	TE	T	NIL
6	M	Atopic eczema	—	+	—	77	T	T	NIL
7	F	Eczema herpeticum	—	—	Me	NT	PTDaE	T	NIL
8	M	? Eczema	—	+	—	NT	PTDaE	T	NIL
9	M	Light sensitive eczema	—	+	Nystatin	{ 83A 80	PT	P	NIL
10	M	Psoriasis	+	—	—	NT	S/all	NIL	NIL
11	M	Psoriasis	+	—	—	83A	PT	T	NIL
12	M	Atopic eczema	—	+	—	80/47/53/54/77/83A/84/88	PT	T	NIL
13	M	Mycosis fungoides	—	+	—	80	PT	T	NIL
14	F	Eczema	—	+	—	{ NT 80/81	T PT	T NIL	NIL 80/81 darker than NT strain

NT, not typable; R/T, resistant to tetracycline; S/all, sensitive to all antibiotics tested.

* All strains were initially tested for resistance to Penicillin (P), Tetracycline (T), Erythromycin (E), Neomycin (N), Dalacin (Da), Gentamicin (Cn), and Methicillin (Me).

Two patients (9 and 14) carried two strains one of which did not vary.

Table 2. *Distribution of resistance variants and number of isolates tested*

Patient	Date	Labile resistance	No. of isolates tested			Percentage with resistance pattern			% R skin % R nose
			Nose	Skin*	Perineum	Nose	Skin*	Perineum	
1	27/Jul/73	P	40	268	NSA	10	95	—	9.50
3	19/Apr/74	P	234	926	88	9	5	7	0.56
re-admit	7/Nov/75	P	160	485	NSA	92	100	—	1.08
	8/May/75	P	298	625	201	5	91	82	18.20
	11/Jun/75	P	165	660	NSA	98	68	—	0.69
	18/Jun/75	P	200	104	NSA	98.5	97	—	0.98
	19/Jun/75	P	632	397	NSA	97	97	—	1.00
	11/Jul/75	P	84	128	NSA	90	99	—	1.10
	5/Aug/75	P	224	152	NSA	99.5	100	—	1.00
2	2/Aug/73	T	76	500	NSA	81	89	—	1.10
	1/May/74	T	377	245	161	14	89	97	6.36
4	8/Oct/74	T	298	339	NSA	10	84	—	8.40
5	30/Sep/74	T	295	743	66	42	87	97	2.07
re-admit	19/Dec/74	T	331	392	NSA	82	96	—	1.17
6	18/Feb/75	T	272	467	NSA	20	41	—	2.05
7	11/Apr/75	T	296	160	NSA	97	86	—	0.89
10	23/Jun/75	T	128	231	160	3	13	100	4.33
	25/Jun/75	T	112	144	ND	1	4	—	4.00
11	4/Jul/75	T	112	40	NSA	100	97	—	0.97
	9/Jul/75	T	128	137	NSA	98	97	—	0.99
	16/Jul/75	T	120	128	8	99	84	100	0.85
12	14/Aug/75	T	136	585	NSA	1	7	—	7.00
	8/Sep/75	T	112	624	72	100	100	100	1.00
13	3/Sep/75	T	42	465	ND	84	56	—	0.67
	11/Sep/75	T	16	496	NSA	75	65	—	0.87
14	7/Nov/75	T	136	379	128	46	83	100	1.80
	27/Nov/75	T	119	212	204	10	87	88	8.70
	30/Jan/76	T	33	381	ND	42	53	—	1.26
	3/Feb/76	T	69	267	ND	19	73	—	3.84
	9/Feb/76	T	117	144	ND	9	60	—	6.67

ND, Not done; NSA, No *S. aureus*; P, Penicillin; T, Tetracycline.

* All skin sites other than the nose and perineum are combined in this group. There was no discernable difference in the various sites.

nose possess twice as many resistant variants as the skin. On the other 18 sampling days the ratio lay between these values. This suggests that the skin tends to select resistant variants (or that the nose tends to select sensitive variants).

Because the distribution of resistant and sensitive variants might result from differences in selection pressure at different sites, an attempt was made to investigate some of the factors which might be relevant.

Plasmid loss in vitro

Plasmid loss was examined after growth at 43 °C and by the use of ethidium bromide or dithranol. The results (Table 3) show that strains from five out of six patients had sensitive variants, usually in small numbers. However, when these strains were stored at room temperature for 3 to 4 months on blood agar base slopes, loss of resistance occurred at higher frequencies. Loss of resistance under these circumstances was noted originally by Barber (1949). Little loss was induced by dithranol, although ethidium bromide and growth at 43 °C were effective. The sensitive variants were always of the same phage type as that of the parent strain.

Table 3. *Plasmid loss in vitro*

	Control	Ethidium bromide at 37 °C	Dithranol at 37 °C	Growth at 43 °C	Ethidium bromide at 43 °C	Storage on Bijou slope
Strain from patient 2						
No. tested for altered pigment	13400	3940	23980	13000	ND	ND
No. of altered pigmented variants	0	0	0	0	—	—
No. tested for R/T	ND	ND	ND	296	360	ND
No. of sensitive variants	—	—	—	0	0	—
Strain from patient 3						
No. tested for R/PT	1746	2909	ND	871	ND	88
No. of sensitive variants*	0	60 S/P 1 S/T	—	1 S/T	—	8 S/P 4 S/T
Strain from patient 5						
No. tested for R/T	960	1046	176	568	752	224
No. of sensitive variants	0	2 S/T	2 S/T	0	1 S/T	105 S/T
Strain from patient 9						
No. tested for R/PT	1160	1432	896	584	ND	211
No. of sensitive variants	4 S/T	32 S/P 1 S/T	0	8 S/T	—	15 S/T 12 S/P
Strain from patient 13						
No. tested for R/PT	1304	1128	944	239	ND	280
No. of sensitive variants	0	12 S/P	1 S/P	1 S/P	—	22 S/P
Strain from patient 14						
No. tested for R/PT	884	448	2000	271	ND	112
No. of sensitive variants	0	1 S/P	0	3 S/T	—	4 S/P

ND, Not done; R/PT, resistant to penicillin and tetracycline; R/P, S/P, resistant or sensitive to penicillin; R/T, S/T, resistant or sensitive to tetracycline.

* All variants had same phage type as parent strain.

Plasmid loss *in vitro* may not accurately represent events *in vivo*. Patient 14 carried two strains of *S. aureus* for most of her stay in hospital. One was a golden pigmented phage type 80/81 strain resistant to penicillin and tetracycline (R/PT) and apparently acquired in hospital; it did not appear to produce variants *in vivo* (in 460 colonies tested, no variants were found). The other was a non-typable, pale-pigmented strain with variable resistance to tetracycline (all were penicillin sensitive). Antibiotic-sensitive variants obtained from the golden R/PT strain *in vitro* retained pigmentation and sensitivity to phages 80 and 81. This also suggests that the pale penicillin-sensitive strain was not derived from the pigmented resistant 80/81 strain.

Pigmentation

Pigmentation might be important in two ways. The major pigments in *S. aureus* are carotenoids which might protect the organism against sunlight. However, this seems unlikely to be significant here because the perineum is rarely exposed to direct sunlight.

Table 4. *Percentage survival of staphylococci on pig epithelium after 7 days at room temperature with uncontrolled humidity: the skin was saturated with synthetic sweat, or synthetic sweat and sebum*

Strain from patient no.	Survivors (%)		
	Skin plus sweat		Skin plus sweat and sebum
2 R/T dark	39 ^a	8 ^e	41 ^a
S/T light	10 ^a	4 ^e	7 ^a
3 R/P	12 ^b	—	—
S/P	54 ^b	—	—
5 R/T	49 ^e	62 ^e	37 ^c
S/T	62 ^c	19 ^e	34 ^c
6 R/T	27 ^d	—	20 ^d
S/T	28 ^d	—	35 ^d
9 R/P	22 ^b	—	—
S/P	23 ^b	—	—
12 R/P dark	21 ^b	—	—
R/P light	23 ^b	—	—
14 R/P	59 ^b	—	—
S/P*	45 ^b	—	—

—, Test not done. *a*, *b*, *c*, *d*, *e*, represent separate experiments, thus conditions were identical for all figures marked *a*, etc. Other abbreviations as in Table 3.

* Obtained during experiments *in vitro* from 80/81 R/PT strain.

Squalene is a precursor of carotene and may form about 10 % of human sebum; the ability of an organism to utilize squalene may be of benefit to it. However, difference in pigment production between the sensitive and resistant variants was only rarely detectable, either visually or spectrophotometrically, and culture in the presence of squalene depressed pigmentation in seven out of eight strains tested.

Survival on desiccation

The survival on drying, on pig skin saturated with synthetic sweat solution, may not closely represent survival on human skin under normal conditions. However, other experimental models are probably even further from reality. No consistent pattern emerged from these studies (Table 4), even sebum was not uniformly inimical to survival.

DISCUSSION

The nose and perineum are the usual sites of growth of *S. aureus* in persons without skin disease but in the patients described here, the number of organisms that were recovered and the frequency of recovery suggested that the organisms were resident and multiplying on several parts of the skin surface. Resistant variants were found more often on the skin surface (including the perineum) than in the nose.

This distribution could be the result either of selection for resistance on the skin or for sensitivity in the nose; the former seems more likely if it is assumed that carriage and replication of a plasmid imposes a 'load' on the organism in most environments; i.e. plasmids would be expected to be maintained only where they are 'necessary'. It is possible that the different growth rates in habitats might account for the differences in distribution (strains lacking plasmids growing faster in the nose). This would be difficult to test experi-

mentally *in vivo* unless a labelled strain could be introduced; the difficulty of establishing *S. aureus* in carrier sites without the help of antibiotics or occlusion would militate against success. Acquisition of plasmids by staphylococci is a remote possibility here, though it can occur experimentally on the skin surface (Lacey, 1975), and transfer of plasmids may also theoretically occur between coagulase-positive and coagulase-negative cocci (Yu & Baldwin, 1971).

Grinsted & Lacey (1973) reported that deeply pigmented strains survived drying and exposure to linoleic acid better than non-pigmented variants. Lacey (1972) had previously reported that antibiotic-resistant variants showed no difference in survival on glass. In the present series, there were no striking differences in pigmentation, though two patients (2 and 12) had strains with slight differences; no consistent difference in survival could be related to pigmentation. This is perhaps not unexpected since only cells with no pigmentation at all were found to have reduced survival capacity by Grinsted & Lacey (1973). In the present series, most variants had apparently identical pigmentation to the parent strain and no differences in survival on drying could be found. Squalene may constitute about 10 % of human sebum (Noble & Somerville, 1974) and dehydrosqualene can accumulate in *S. aureus* strains that have lost the ability to synthesize complete carotenoid pigments (Suzue, Tsukada & Tanaka, 1968). Utilization of squalene may be expected to be associated with high pigmentation. However, squalene depressed pigmentation in most of the isolates tested. The incorporation of glycerol monoacetate into agar enhanced pigmentation equally in parent and variant isolates.

No attempt was made in these experiments to examine the remainder of the skin flora for antibiotic production (in most patients *S. aureus* was the predominant member of the flora). Amongst the resident skin flora, members of the Micrococcaceae produce antibiotics (Selwyn, 1975) as do dermatophyte fungi (Youssef *et al.*, 1976). The contribution of these organisms to the ecology of *S. aureus in vivo* is unclear. The therapeutic antibiotic exposure of these patients seems unlikely to account for the distribution observed, as none had been treated with tetracycline or penicillinase-vulnerable penicillins.

Factor(s) that select or maintain the fully resistant parent organism on the skin are thus obscure. Antibiotic resistance, though easily measured, is unlikely to give the cell an advantage *in vivo* here. However, the penicillinase plasmid may carry other information. For example, in *Staphylococcus epidermidis* the penicillinase plasmid may also control the uptake of mannitol and β -glucosides, ribose fermentation and phospho- β -glucosidase activity; phage absorption, restriction and modification and growth factor requirements are also associated with penicillinase production (Schaeffer, 1972). In *S. aureus*, resistance to cadmium and mercury may be closely associated with penicillinase production but even exposure of people to mercury may not result in the selection of resistant strains of staphylococci (Groves *et al.*, 1975).

Further examination of the ecological pressures that result in the selection or maintenance of resistant or sensitive variants on the skin and nose are required.

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