CARRIAGE OF INHIBITOR-PRODUCING ORGANISMS ON HUMAN SKIN

W. C. NOBLE AND JEANETTE A. WILLIE

Department of Bacteriology, Institute of Dermatology, Homerton Grove, London E9 6BX

SELWYN (1975) described the carriage of antibiotic-producing organisms on human skin and showed how they might protect the carrier against colonisation with potential pathogens such as *Staphylococcus aureus*. Subsequent work by Selwyn and his colleagues (Marsh and Selwyn, 1977*a* and *b*; Milyani and Selwyn, 1978) has shown that these antibiotic producers can restrict the growth of susceptible strains *in vitro*. This paper reports a study of the carriage, in patients with skin disease, of ‘antibiotic’-producing cocci with an activity against *S. aureus*.

MATERIALS AND METHODS

Samples for bacterial culture were taken from the nose, the chest, the perineum and one or more lesions of patients admitted to the Inpatient Department of St John's Hospital for Diseases of the Skin; cotton-tipped swabs moistened with broth were used. When *S. aureus* was isolated from a skin lesion the opinion of a clinician, usually the senior house officer, was sought on whether the lesion was clinically infected or only colonised; these opinions were recorded. The swabs were inoculated on to Blood Agar Base (Oxoid CM55) used as a ‘nutrient’ agar and the plates were incubated aerobically for 24 h at 37°C and left on the bench for a further 24 h at ambient temperature. A semi-quantitative assessment was made of each colony type present.

Representatives of all colony types were subcultured on to blood-agar-base plates under a serial code number and examined for production of inhibitors. In the initial studies on about 4000 isolates, the method used was a modification of a pyocine typing method (Darrell and Wahba, 1964) in which test strains were inoculated as a heavy streak across the diameter of a glass petri dish containing blood-agar-base medium. This plate was then incubated for 24 h, after which the growth was removed by scraping with a microscope slide and the plate was exposed to chloroform vapour for 10 min. After exposure to air to permit evaporation of residual chloroform, four indicator strains were inoculated at right angles to the site of the original culture. The plates were then reincubated for 24 h at 37°C. Antibiotic production was indicated by a failure of growth in the area from which the test strain had been removed.

Another 1300 isolates were tested by a modification of this method in which, after removal of the test-isolate growth with a glass microscope slide, any remaining cells were spread over the surface of the medium with a cotton tipped swab (‘Q-tip’) moistened in broth. The plate was then exposed to ultraviolet light for 20 min. to kill remaining cells; four indicator strains were then applied as previously described.

The indicator strains used were *S. aureus* PS80, the original epidemic strain which remains virulent for mice and is used for propagating phage 80; *S. aureus* PS 42E, the propagating strain for phage 42E; *S. aureus* Wood 46, the standard α -haemolysin producer; and *Micrococcus luteus* 27B, a wild-type isolate from a patient.

RESULTS

From 292 patients, 5282 strains were tested. Inhibitor producers active against *S. aureus* were found in about 5% of sites (table I) or about 10% of persons. Only 1.6% of strains tested were producer organisms. The prevalence of producer organisms was approximately the same

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TABLE I
Isolation of "antibiotic"-producer organisms from carrier sites and lesions

Site	Number of persons sampled*	Number (and percentage) yielding producer organisms
Nose	260	8 (3)
Chest	292	17 (5.5)
Groin	291	14 (4.5)
Lesion	295	24 (8)

* Overgrowth of plates by *Proteus* resulted in loss of some samples.

in lesions that were only colonised and those that were clinically infected with *S. aureus* (table II).

Table III shows that the proportion of skin lesions yielding antibiotic producers was only slightly greater in those yielding no pathogens (*S. aureus*, *Streptococcus pyogenes*, *Proteus* or *Pseudomonas*) than in those yielding *S. aureus* and other pathogens.

When present, the producer organisms were the dominant flora in 30% of cultures and a minority flora in 25%; in the remainder the producer and other organisms were present in about equal numbers. There was no difference in the proportion of producer organisms identified by the two methods.

TABLE II
Colonisation and clinical infection with Staphylococcus aureus in relation to presence or absence of antibiotic producers in skin lesions

Antibiotic producers	Number (and percentage) of skin lesions	
	colonised only	clinically infected
present	5 (7.4)	6 (7.2)
absent	63	77
Total	68	83

DISCUSSION

The prevalence of inhibitor producers in the isolates examined in this series appears to be low. Hsu and Wiseman (1967) reported that 4.9% of 1065 isolates of *S. aureus* and 8.5% of 387 coagulase-negative cocci produced antibiotics active against the Oxford strain of *S. aureus*. In contrast, Selwyn (1975) found that isolates from 22.6% of 340 persons produced antagonists when tested against coagulase-negative staphylococci and coryneforms (Selwyn and Ellis, 1972). Holland, Cunliffe and Eady (1979) found antagonists produced by skin Micrococcaceae in

TABLE III
Presence of S. aureus and other pathogens in relation to presence or absence of antibiotic producers

Antibiotic producers	Number (and percentage) of skin lesions	
	yielding <i>S. aureus</i> or other pathogens	not yielding <i>S. aureus</i> or other pathogens
present	9 (7.1)	15 (8.3)
absent	118	166
Total	127	181

15/148 (10%) isolates tested from 44 persons but by 38/93 (41%) of *Propionibacterium acnes* from the same individuals. However, only 2.7% of the Micrococcaceae would inhibit other Micrococcaceae, whereas 41% of the *P. acnes* were active against other *P. acnes*. Holland and his colleagues reported that media containing 0.5% glucose, such as that used by Selwyn, can lead to the appearance of inhibition solely as a result of acid production. They are careful to emphasise that not all "inhibition" or "antagonism" observed on agar medium is the result of antibiotic production. The low rates of producer strains in the present series and in those of Hsu and Wiseman (1967) and Holland *et al.* (1979) when compared with the findings of Selwyn (1975) thus seem referable to the more stringent test conditions when media containing only small amounts of glucose are used. Antibiotics produced by Micrococcaceae are polypeptides (Halbert, Swick and Sonn, 1953; Hsu and Wiseman, 1972).

Selwyn (1975) was able to show a reduction in colonisation by pathogens of lesions containing producer organisms, but in this series no difference in colonisation or in clinical infection could be detected. Experimental studies of our own (unpublished data) show that inhibition, presumably due to antibiotic production, is caused by producer organisms against indicator strains on the skin surface of the hairless/obese mouse (J. Atkinson, in preparation) and in a subcutaneous model of infection (Noble, 1965). This confirms the observations of Selwyn and his colleagues on in-vitro antagonism in various experimental situations (Marsh and Selwyn, 1977*a* and *b*; Milyani and Selwyn, 1978). Dr T. Sethna and Dr S. Selwyn (personal communication) have demonstrated in-vivo antagonism. Nevertheless it is difficult to assess the likelihood of antibiotic production *in vivo* in the human host. If production occurred it would seem to imply that producer organisms should be the dominant form, because producer strains should outgrow non producers, yet this occurred in only 30% of instances in this series. Youssef *et al.* (1979) showed that dermatophyte fungi produced detectable amounts of antibiotic, in this case penicillin-like substances, in only 4 of 45 patients, although these four strains represented 16% of the 25 fungi able to produce such antibiotics *in vitro*. In Youssef's series temperature was found to be of importance in antibiotic production *in vitro*. We do not yet know the precise conditions in which organisms grow at the skin surface; such knowledge would be of immense value in assessing the skin as a microbial habitat and in deciding whether antibiotic production *in situ* could be put to therapeutic use.

SUMMARY

The carriage on skin lesions, on healthy skin and in the nose, of micro-organisms that produce antibiotics active against *Staphylococcus aureus* has been studied. About 5% of sites (10% of all patients) yielded such organisms. No evidence could be found that the presence of producer organisms in skin lesions had a protective effect against colonisation by *S. aureus*.

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