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Ecological effects of antibiotic production by dermatophyte fungi

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SUMMARY

Antibiotic production by dermatophyte fungi has been demonstrated *in vivo* in the lesions of patients with dermatomycoses. Patients infected with antibiotic-producing strains more frequently carried cocci resistant to penicillin and other antibiotics than did patients infected with non-producer strains. The total bacterial load was less in lesions caused by producer fungi. *In vitro* studies demonstrated the selection of penicillin-resistant *S. aureus* from mixed populations of resistant and sensitive cells.

INTRODUCTION

In 1978 Youssef *et al.* described the production by dermatophyte fungi of several commonly used antibiotics; these included penicillin, 6 amino-penicillanic acid and streptomycin-like antibiotics as well as a number of unclassified substances produced by representatives of the species *Trichophyton mentagrophytes*, *T. rubrum* and *Epidermophyton floccosum*. The paper dealt only with production *in vitro*, though besides growth in a fluid medium designed to facilitate production of penicillin, growth and production was obtained on human stratum corneum, especially when a synthetic sweat solution was used as nutrient supplement. Attention was drawn to the possibility that antibiotics might be produced *in vivo*, that is in the natural lesion of tinea, and that this might have two consequences: one the selection of a resistant flora and the other the induction of allergy to the antibiotic. In this paper we demonstrate the production of antibiotic *in vivo* and describe the natural effect of this on the skin flora; experimental ecological studies are also described.

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MATERIALS AND METHODS

In vivo *studies*

Patients attending the outpatient department of St John's Hospital for Diseases of the Skin with suspected dermatomycosis were studied. Conventional skin scrapings were taken to establish the diagnosis by microscopy and culture (this was carried out by members of the Department of Medical Mycology). When microscopy was positive samples were taken by pressing Steridrape (3M Co. Ltd) across the lesion so that infected epithelium adhered to the sticky surface. One such sample was laid directly on the surface of a petri dish containing agar seeded with spores of *Bacillus subtilis* (NCTC 8236) and the other was attached to a microscope slide and incubated in a humid atmosphere in a petri dish as described by Knight (1973) for 7 days at 30 °C, and then laid on a plate seeded with *B. subtilis* spores. After overnight incubation at 37 °C these plates were examined for zones of inhibition which indicated the presence of antibiotic. Conventional bacteriological samples were also obtained using a soluble alginate swab which was dissolved in citrate/hexametaphosphate buffer and inoculated on blood agar plates (Oxoid blood agar base containing 7% horse blood) and on Oxoid CLED medium and incubated at 37 °C overnight. Colony counts were made by the method of Miles & Misra (1938). Organisms recovered from these samples were identified using the tests described by Cowan & Steel (1974) and all were tested for resistance to the following antibiotics: penicillin G, 10 units; ampicillin, 10 units; methicillin, 10 μg; fusidic acid, 10 μg; streptomycin, 10 μg; tetracycline 30 μg, using Oxoid sensitivity disks on Oxoid DST agar.

At the time of sampling, notes were made of the age, sex and race of the patient together with the extent, site and duration of the lesion plus any history of previous treatment.

Fungi recovered from the clinical samples were received from the Medical Mycology Department and examined for antibiotic production in fermentation unit medium in shake culture in the way described by Youssef *et al.* (1978).

In vitro *studies*

Sterile porcine epithelium (Lyoderm, Armour Pharmaceutical Co. Ltd) was cut into oblong pieces of about 0·5 cm^2 area and reconstituted in synthetic sweat solution (Murphy, 1975). Normal human stratum corneum was collected on Steridrape and sterilized by ethylene oxide gas. Human and porcine epithelium was attached to microscope slides and kept in a humid atmosphere in a petri dish (Knight, 1973), it was then seeded with a known antibiotic-producing dermatophyte or a strain in which antibiotic production had not been detected and with a mixture of penicillin-sensitive and -resistant cells of *Staphylococcus aureus*. The ratio of resistant to sensitive cells was about 1:5. The penicillin-sensitive strain was derived from the resistant parent by loss of the penicillinase plasmid (Noble, 1977), both variants were resistant to tetracycline and of phage type 83A. After incubation at 30 °C for 7 days to permit growth of the dermatophyte, the skin samples were shaken in 2 ml of buffer pH 8 containing 1% Tween 80 to

suspend the bacterial cells, and colony counts were made on agar with penicillin at 30 units/ml or 2·5 units/ml or without penicillin.

Cultures of the penicillin-resistant variant of the *S. aureus* strain, a wholly sensitive strain of *Micrococcus luteus*, and a strain of *Candida albicans* were studied for their ability to grow on porcine epithelium in the absence or presence of a *T. mentagrophytes* strain known to produce penicillin-like and streptomycin-like antibiotics in fermentation unit medium at 30 °C. Incubation was at 30 °C for 7 days. Bacteria and yeasts were inoculated in 5 μl amounts at one end of the strip of epithelium as a fluid suspension. Dermatophytes were inoculated in 5 μl amounts as a suspension of spores. Growth was assessed as follows: bacteria and yeasts could be seen to produce visible colonies all over the piece of epithelium (score 3), in the centre of the strip (score 2) or only at the site of inoculation (score 1); dermatophyte growth was assessed on a scale based on that of Knight (1973), production of long hyphae giving a net-like mycelium (score 2), production of short hyphae only (score 1) or no germination (score 0).

RESULTS

Full data were available on 49 patients with proved dermatophytosis. Five patients were infected with *T. mentagrophytes*, 35 with *T. rubrum* and 5 with *E. floccosum;* a further 4 lesions failed to yield viable fungus and were not considered further. Inhibition of *B. subtilis* was obtained with direct Steridrape samples from 7 of these 45 patients but 3 of these had a history of treatment with various antibiotics; at least 4 of the 45 therefore showed evidence of antibiotic production *in vivo*.

Twenty-five of the 45 fungi isolated were shown to produce antibiotics *in vitro* in shake cultures of fermentation unit medium (4 *T. mentagrophytes*, 19 *T. rubrum* and 2 *E. floccosum*). Seven strains were classified as 'poor' producers (less than 0·8 units/ml equivalent penicillin) and four of these appeared to produce only penicillin-like substances. The remaining three poor producers and all good producers formed penicillin and other antibiotics not destroyed by penicillinase. Six strains produced antibiotic after 7 days' incubation on the Steridrape sample. In all, 7 strains showed the ability to produce antibiotic on stratum corneum from patients, 4 of these on direct testing (1 *E. floccosum*, 1 *T. mentagrophytes*, 5 *T. rubrum*).

No relation between antibiotic production and age, sex, race of the patient, site or duration of the lesion could be found.

Bacterial growth was obtained in 44 of the 45 samples (the remaining sample was from an elderly man who had received antibiotic treatment). The principal bacterial flora was of coagulase-negative cocci, both *Staphylococcus* spp. and *Micrococcus* spp.; *S. aureus* was recovered from three patients only and Gram-negative rods were also recovered on three occasions. Quantitative counts showed that lesions which yielded an antibiotic-producing fungus had lower bacterial counts than those with a non-producer (Table 1).

Table 1 also shows the relation between the ability of the dermatophyte fungus

Table 1. *Relation of antibiotic production by dermatophytes in relation to the number of bacteria in the lesions and the antibiotic resistance patterns of those bacteria*

Dermatophyte	Total samples	% with viable bacterial count less than 5×10^3	% with principal bacterial flora resistant to:* P	A	T	F	S	M
Antibiotic producer	25	92	84	80	68	56	36	16
Non-producer	20†	60	47	47	26	26	16	16
Total lesions†		45	44	44	44	44	44	44
χ^2		6·6	6·7	5·1	7·4	3·9	2·2	0·005
P		0·01	< 0·01	< 0·025	< 0·01	< 0·05	N.S.	N.S.

* P, benzyl penicillin; T, tetracycline; F, fusidic acid; S, streptomycin; A, ampicillin; M, methicillin.
† One lesion yielded no bacteria.

Table 2. *Recovery of penicillin-resistant cells of* S. aureus *before and after incubation on epithelium with dermatophyte fungi*

(Three replicas of three experiments each have been pooled in constructing the cells of this table.)

		Before incubation: Total cells counted at dilution 10^{-1}	R/P (%)	After incubation: Total cells counted at dilution 10^{-3}	R/P (%)	χ^2	P
T. mentagrophytes penicillin producer							
No. 7	Human stratum corneum	125	12·8	463	28·7	13·2	< 0·001
	Pig skin	139	23·0	433	34·2	6·1	< 0·02
No. 8	Human stratum corneum	166	19·3	498	31·5	9·2	< 0·01
	Pig skin	188	18·6	541	32·0	12·2	< 0·01
T. rubrum							
Non-producer	Human stratum corneum	125	12·8	459	16·3	0·9	NS
No. 20	Pig skin	174	21·3	394	21·6	0·01	NS

R/P = resistant to penicillin.

to produce an antibiotic and the resistance pattern of the most numerous bacterium in the lesion. There were significantly more penicillin-resistant strains, tetracycline-resistant strains and fusidic-acid-resistant strains in lesions caused by producer fungi.

Table 2 shows that the ability of the fungus to produce penicillin on stratum corneum leads to an increase in the proportion of penicillin-resistant cocci in mixed culture. The cultures made using a fungus which was a non-producer, even in fermentation medium, caused no shift in the balance of penicillin resistance. The change in resistance therefore seems likely to have been related to penicillin

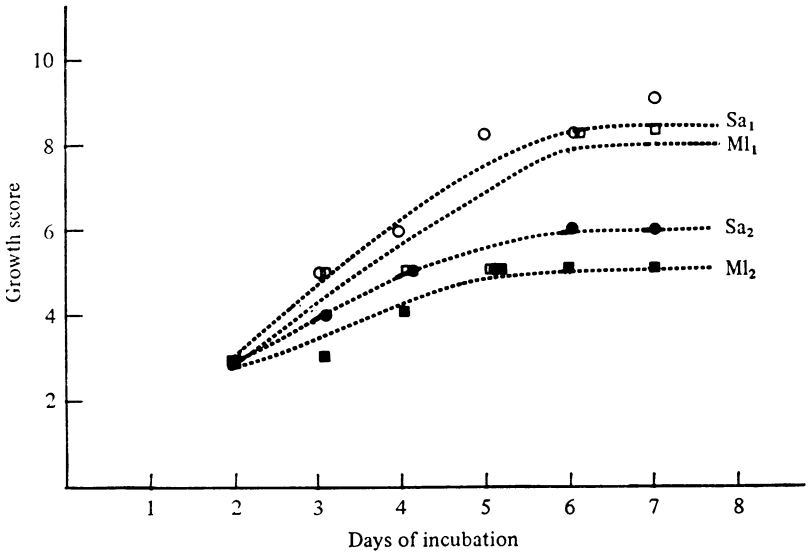

Fig. 1. Growth on porcine skin of *Staphylococcus aureus* (Sa) and *Micrococcus luteus* (Ml) in the absence (1) and presence (2) of antibiotic-producing strain of *Trichophyton mentagrophytes*.

production. All staphylococcal counts were about $3{\cdot}5 \times 10^2$ higher after incubation.

Cultures of *S. aureus*, *M. luteus* and *C. albicans* grew well on porcine stratum corneum in the absence of the dermatophyte. In its presence, however, growth of both *S. aureus* and *M. luteus* was depressed although the dermatophyte grew normally (Fig. 1). When mixtures of the yeast and dermatophyte were incubated together, the growth of both was depressed.

DISCUSSION

Production of antibiotic *in vivo* seems certain in 4 of the 45 patients studied; 25 strains of dermatophyte were shown to be capable of antibiotic production and these 4 instances therefore represent 16% of the potential. This low incidence may reflect the small amounts of antibiotic produced and the insensitive detection methods used, or it may reflect the true *in vivo* production. Further studies are needed on this point.

Uri, Szathmary and Herpay (1957*a*, *b*) demonstrated penicillin production in fragments of skin or hair from 21 of 25 patients infected with dermatophytes. They reported that all producers were either *T. mentagrophytes* or *E. floccosum* and that non-producers were all *T. violaceum*. Smith & Marples (1965) reported that skin from rabbits infected with the hedgehog ringworm fungus *T. mentagrophytes* var. *erinacei* also showed evidence of penicillin production. *In vitro* these authors found antibiotics to be produced by five of five *T. mentagrophytes* and from the only *E. floccosum* tested, but not from a single *T. rubrum* tested (only one *T. mentagrophytes* isolate was tested *in vivo*). Both groups of workers

refer to the production of unknown antibiotic substances not destroyed by penicillinase. During the present studies (this paper and Youssef *et al.* 1978) antibiotics have been detected *in vitro* from 12 of 13 *T. mentagrophytes* isolates, 26 of 46 *T. rubrum* and 11 of 18 *E. floccosum*. All antibiotic producers formed penicillin whilst other antibiotics were produced by 10 *T. mentagrophytes*, 18 *T. rubrum* and 11 *E. floccosum*. *In vivo* antibiotic production was found only in *T. rubrum* isolates.

Although, as expected, more penicillin-resistant organisms are found in lesions caused by fungi shown to produce penicillin *in vitro*, some comment is perhaps needed on the other antibiotics. Tetracycline and fusidic acid were not found to be produced by these fungi but there was a significant excess of resistance to these antibiotics in lesions caused by producer strains. It seems most probable that this is a reflexion of the fact that resistance to tetracycline and fusidic acid is most common in strains that are also resistant to penicillin and it was selection for penicillin resistance that caused the excess. Streptomycin and methicillin resistance are also found most frequently in penicillin-resistant cocci, but the prevalence of resistance is low in organisms from outpatients. Ampicillin resistance closely follows that for benzyl penicillin because of the preponderance of Gram-positive cocci, the sole difference occurring with a Gram-negative rod.

These results confirm the observations made previously (Wallerström, 1968; Youssef *et al.* 1978) that selection of a resistant flora occurs as a result of dermatophyte infection and shows that the observation can be refined to reveal greater resistance in lesions caused by dermatophytes known to be antibiotic producers. Youssef *et al.* (1978) reported that 57% of patients from whom fungus could be grown carried a penicillin-resistant coccus compared with 38% from whom no fungus was grown. The present study shows that 47% of lesions with non-producer fungi had penicillin-resistant organisms but that 80% of the 25 lesions harbouring producer fungi did so. Bibel & LeBrun (1975) showed how experimental dermatophyte lesions could result in the selection of a resistant flora and the present study illustrates the selection of penicillin-resistant *S. aureus* from a mixed population of sensitive and resistant cells *in vitro*. No selection occurred when a non-producer strain was used

When a dermatophyte known to produce both penicillin and streptomycin-like antibiotics *in vitro* was grown with a penicillin-resistant but streptomycin-sensitive *S. aureus* the latter was inhibited. Thus the production of other antibiotics may account for the lower yields of viable cells in producer-fungus infected lesions despite the availability of penicillin-resistant cocci.

The mutual suppression of growth when *C. albicans* and *T. mentagrophytes* were incubated together on porcine epithelium has no obvious explanation although King *et al.* (1976) identified carbon dioxide produced by *C. albicans* in mixed culture as inhibiting the growth of dermatophytes and inducing arthrospore formation. An alternative may be competition for some essential nutrient; for example, Littman & Miwitani (1963) reported biotin as essential to the growth of *C. albicans*. Natural mixed infections with dermatophytes and *Candida albicans* are rare though other yeasts are sometimes associated with dermatophytes (e.g. Schönborn, 1968).

It was noticeable that mixed cultures of dermatophyte and *S. aureus* caused more complete dissolution of the porcine epithelium than did either strain alone. Both staphylococci and dermatophytes have well-characterized proteolytic enzymes (Minocha *et al.* 1972; Arvidson, Holme & Lindholm, 1972); the existence of specific keratinolytic enzymes is a matter for debate centring on the nature of keratin itself. It has been reported that clinical lesions infected with both organisms may be more severe than those infected only with fungus (Marples & Bailey, 1957).

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