Tropical Otomycosis

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Introduction

The Aden monsoon season provides an excellent opportunity for observing a European population living as a compact social group in a tropical climate. During the period of our investigation 221 patients suffering from otorrhoea were studied with a special reference to fungus infections occurring in tropical conditions. All the cases to be described occurred in Europeans, serving members of Her Majesty's Armed Forces and their dependants. The population at risk during this period was in the region of 30,000 persons. The monsoon season in Aden (April–November) is characterized by high daily shade temperatures averaging between 95–105° F and a relative humidity of 80–90 per cent. There was little or no recorded rainfall at this time. These climatic conditions are particularly favourable for the growth and dissemination of fungal organisms.

Fungi are vegetable micro-organisms. They are members of the plant kingdom which exhibit no differentiation into root, stem or leaf and contain no chlorophyll. The absence of chlorophyll condemns them to a saprophytic or parasitic existence. They are, however, not exacting in their growth requirements and are readily cultured on simple media.

Fungi abound in any soil or sand which contains decomposing vegetable matter, present in the droppings of cattle, goats, dogs and other domestic animals. This is desiccated rapidly in the tropical sun and dispersed as wind blown particles. The airborne fungal spores are carried on droplets of water vapour, a fact which we believe correlates with the higher incidence of infection during the monsoon when the relative humidity rises above 80 per cent. Austwick and Ayerst (1963) have shown that *Aspergillus flavus* required warm humid conditions for growth, while experimentally they found that the fungus would not grow at a relative humidity below 80 per cent. and the optimum temperature for growth was 33° C (90° F).

The class fungi Imperfecti contain many fungi of medical importance. Amongst these are the genera Aspergillus and Candida. These fungi are, however, dissimilar. Aspergilli are filamentous fungi exhibiting hyphae which branch and intertwine to form a dense mass of growth, the mycelium. Projecting from the mycelium are the sporulating hyphae or conidiophores which bear the sexual reproductive bodies, conidiospores.

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Candida, however, are yeast-like fungi growing both as yeasts, reproducing by budding and as long filamentous cells joined end to end. These are the pseudohyphae. Both forms may be seen under the same environmental conditions.

For the purpose of this report we are interested particularly in fungi that exist parasitically on skin and its appendages in the external auditory meatus.

The external auditory meatus has a lining of skin similar to that of other parts of the body. However, it differs in the following respects:

(1) It is only exposed to the atmosphere by the small meatal inlet and therefore represents a skin lined culture tube which provides optimum conditions for fungal and bacterial growth.

(2) The skin has a very rigid support as it is closely applied to the perichondrium and periosteum forming its margins, so there is little room for lateral expansion and oedema is accompanied by intense pain.

(3) The hair follicles, sweat, ceruminous and sebaceous glands are confined entirely to the outer third of the ear canal. Ceruminous glands are modified sweat glands. They differ from the characteristic apocrine type in that the cells are filled with fat droplets and brown pigment granules which form ear wax. Sweat has normally an acid reaction due to the presence of acid sodium phosphate which maintains a pH level of 4·7 to 7·5 (West and Todd, 1961), but this becomes alkaline in the presence of excessive sweating (Macleod and Muende, 1946). For this reason tropical ear wax fails to provide the protective sleeve normally attributed to it in temperate conditions. In addition, the more dilute oliferous form of wax renders it more readily removed by irrigation, which is associated with swimming and diving.

Clinical Study

Patients suffering from otorrhoea referred to the otologist for specialist investigation and treatment fell into the following categories:

1. Cases which failed to respond to treatment in a period of 3 to 4 weeks.
2. Cases which frequently relapsed following apparent response to treatment.
3. Cases with acute symptoms such as meatal furuncles.
4. Cases with otorrhoea complicating a previously recognized otic pathology such as:
   (*a*) Meatal stenosis.
   (*b*) Unhealed perforations of the tympanic membrane.
   (*c*) Mastoid or fenestration cavities.

Patients suffering from acute otitis externa, more commonly termed locally as "swimmers' ear" or "Aden ear" were treated adequately by their Unit Medical Officer and were not included in this series.

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Diagnosis

The criteria used for establishing a diagnosis of otomycosis was a combination of any two or more of the following positive findings:

1. The clinical observation of an active fungal infection in the ear.
2. The demonstration of fungal elements by lactophenol blue staining of aural discharge on a microscopic slide.
3. Grocott's mycotic stain of epithelium removed from the deep meatus.
4. Culture of aural discharge on Sabouraud's glucose agar plates.

Laboratory Techniques

Two aural swabs and a fragment of desquamated deep meatal epithelium was obtained from each patient. One of the swabs was cultured on 2 per cent. blood Agar aerobically and anaerobically at 37° C overnight and examined for bacterial growth. The other swab, together with the desquamated epithelium, was used for mycological examination. Pus from the swabs was digested on a microscopic slide with 10 per cent. potassium hydroxide and then stained with lactophenol blue. This preparation was examined microscopically using the 2/3rds and 1/6th objective. These appearances have been well described elsewhere by Gregson and La Touche (1961).

The material was also cultured on Sabouraud's glucose agar plates at 37° C. The plates were examined after 3 and 10 days' incubation for fungal growth at the site of the inoculum. This was an important provision necessary to exclude a false positive result due to airborne contaminants. It was unnecessary to add antibiotics to this medium, the low *p*H and high glucose content was sufficient to suppress bacterial growth.

The biopsy material was fixed in 10 per cent. formal saline mixture in a conical centrifuge tube, processed through alcohols and embedded in paraffin wax. The block was then sectioned and stained with Grocott's (1955) modification of Gömöri's silver methenamine impregnation method.

The biopsy material was minute, but the technique described enabled a histological preparation to be made from the smallest of fragments which would otherwise have been impossible.

The histological sections were then reported upon by one of us. Our histological criterion for the diagnosis of fungal infection was the presence of spores and hyphae within the tissues. In some cases it was possible to give the genus of the fungus present (Fig. 1).

Aspergilli species were easily identified by the presence of typical conidiophores and Candida species as budding yeasts and occasional pseudohyphae.

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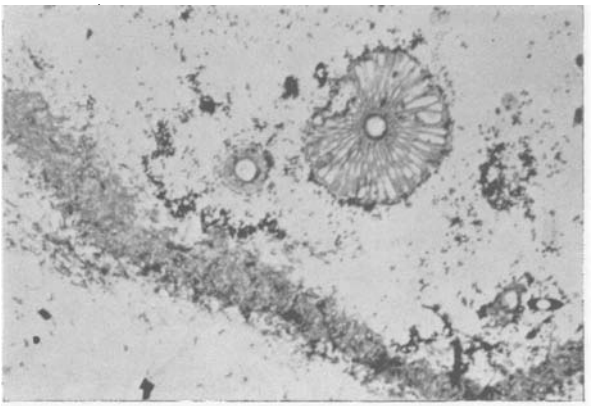


FIG. 1.
This shows two conidiospores and clear evidence of fungal invasion of the green counter-stained epithelium.

Results

Of the 221 patients investigated, a positive diagnosis of otomycosis was obtained in 159. This represents 79 per cent. of the total patients referred.

Further differentiation of the fungal elements demonstrated that 103 cases were due to Aspergillus and 56 were due to infection by Candida.

The Aspergillus could be further divided in the following groups:

A. niger	21
A. flavus	15
A. terreus	14
A. fumigatus	12
A. glaucus	4
A. candidus	1
Unspecified	32

Aspergillus niger was the most common of the Aspergilli isolated. The twenty-one cases cultured represented 20 per cent. of the total. Clinically this group proved to be the most rapid in growth, a fact which was confirmed on culture. Only in three cases were the fungal elements not recognized on initial scrutiny under lactophenol blue staining. A special interest was taken in the association of *Aspergillus niger* with pathogenic bacteria (Table I). In ten (50 per cent.) of the twenty-one patients with *Aspergillus niger* infection no bacteria were demonstrated in culture.

Aspergillus flavus was the second most common group identified. Only in three patients of this group did we fail to recognize the fungus by the direct lactophenol blue staining of the meatal discharge. In seven patients, representing 50 per cent. of the total, the fungal growth was unassociated with pathogenic organisms.

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TABLE I.

To Show the Association Between Fungus and Bacterial Growth in Culture.

Fungus	No. of cases Investigated	Positive Bacterial Culture	Negative Bacterial Culture
A. niger	21	11	10
A. flavus	15	8	7
A. terreus	14	9	5
A. fumigatus	12	8	4
A. glaucus	4	2	2
A. nidulans	4	1	3
A. candidus	1	—	1
Unspecified	32	22	10
Candida Sp.	56	36	20

Aspergillus terreus was cultured from fourteen patients in this series, all of which were detected by lactophenol blue staining of the discharge on the microscopic slide. Five were unassociated with bacteria.

The fourth most common group encountered were the twelve patients with *Aspergillus fumigatus*. Eleven were demonstrated by staining with lactophenol blue. Four were unassociated with any bacterial infection.

The group of unspecified fungal infections consisted of patients in which fungal elements were conclusively demonstrated at the initial clinical examination, by lactophenol blue staining of the slide smear and subsequently Grocott's mycotic staining of epithelial material removed from the deep meatus, but where subsequent culture failed to produce fungal growth at the site of the inoculum on a Sabouraud's plate. Failure to determine the specific Aspergillus involved occurred in thirty-two of the total of 103 patients (30 per cent.). The reason for this appeared to be that positive culture of ear swabbings was more likely to be successful when the fungus was in the sporing phase of its life cycle. Secondly, a knowledge of previous treatment with fungicidal or fungi-static agents by the patient's own Medical Officer was not always available when the case presented for treatment. Such previous therapy may well modify the subsequent culture characteristics of the fungus and can result in many negative cultures.

Table II demonstrates the lack of relationship between specific aspergilli and bacterial species in this series. In no instance was there any statistical correlation between the fungus and bacteria isolated.

Candida was grown from the aural discharge of fifty-six of the total 159 cases. This represented 35 per cent. of the total in this series. Of these, yeast forms were demonstrated in the discharge by Gram staining in a total of 73 per cent. of the cases and subsequent pure culture occurred in twenty-nine cases, i.e. 50 per cent. of the total investigated. The association of Candida with pathogenic bacteria is shown in Table III.

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TABLE II.

To Show the Relationship Between Specific Aspergilli and Bacteria in This Series

	St. Aureus	*Ps. Pyocyaneus*	*E. Coli*	Proteus Sp.	*St. Aureus*/ *Ps. Pyocyaneus*	*E. Coli*/ Proteus Sp.	*St. Aureus*/ *E. Coli*	*Ps. Pyocyaneus*/ *E. Coli*	Proteus Sp./ *Ps. Pyocyaneus*	Totals
A. niger	4	3	2		1	1				11
A. flavus	4	1	1	1	1					8
A. terreus	6	1	1				1			9
A. fumigatus	2	3	2						1	8
A. nidulans	1									1
A. glaucus	1		1							2
A. candidus										—
Unspecified	4	6	6	1	3	1		1		22
Totals	22	14	13	2	5	2	1	1	1	61

TABLE III.

To Show the Association Between Candida and Bacteria in This Series.

	Neg. Bact. Cult.	St. Aureus	Ps. Pyocyaneus	E. Coli	Proteus Sp.	St. Aureus	Ps. Pyocyaneus	E. Coli	Proteus Sp.	Total
Candida	20	11	13	7	—	4		1		56

Treatment

Early in the monsoon season when it was suspected that the number of cases of otomycosis referred to the Department was going to reach epidemic proportions, it was realized that many of the standard fungicidal agents were relatively ineffective when used in tropical conditions. The onset of symptoms in the majority of cases was explosive in nature and characterized by the sudden onset of considerable oedema and pain which demanded immediate maximum therapeutic effort, so that there was no place for conducting clinical trials using less efficient agents. The fungicidal preparations available were:

1. Hibitane.
2. Nystatin.
3. S.7. bis–(2-hydroxy-5-chlorophenyl) (Stride, 1962).
4. Penotrane (phenylmercuric dinaphthylmethane disulphonate) used as Tincture 0·1 per cent. and impregnated ribbon gauze (Ototrane).

It was quickly noted that Hibitane did little to alleviate the symptoms apart from the relief which was due to efficient meatal toilet. In addition, Nystatin was disappointing; it is possible that its potency was modified by the tropical conditions. S.7. was used in only four cases and each associated its use with increased ear pain, which forced them to cease treatment and seek further advice.

Undoubtedly, Penotrane was the most efficient agent encountered and upheld all the manufacturer's claims. Penotrane is a chemical antiseptic effective against both Gram positive and Gram negative bacteria and against fungi such as Aspergillus and Candida. This activity is slightly increased rather than reduced by the presence of serum or pus. (Goldberg *et al.*, 1950). Penotrane penetrates intact epithelium, a particular advantage in the treatment of fungus infection where the mycelia are usually confined to the epidermis.

At the patient's first visit, culture and biopsy specimens were obtained, followed by a careful meatal toilet. This required special attention to the pretympanic sulcus, which is the most common site of early infection; a failure to clean this area may be responsible for subsequent relapses. When all the infected material had been removed from the meatus then a short length of ototrane ribbon gauze was introduced so as to lie adjacent to the tympanic membrane and the meatal wall. Impregnated gauze is the only efficient means of maintaining therapeutic levels of medicaments over the whole of the surface area of the meatus for long periods. It could be introduced in all but the most oedematous external auditory meati and was usually well tolerated, as it moulded itself into the shape of the meatus when warm. It could be left in position for 3 days, after which time it became dry and extruded itself from the ear spontaneously. The degree of deafness produced was minimal and was usually accepted without comment.

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The patient attended the Department for the second time 3 days later, when the gauze was removed and the meatal toilet was repeated. A further strip of ribbon gauze was then reinserted into the ear and the patient instructed to remove this himself in a further 3 days. This procedure is to some extent self-cleansing, removing residual particles of debris. He was then instructed to insert tinct. Penotrane into the ear at intervals during the day until his third visit to the Department after 1 week when the aural toilet could be repeated. By this method relief of acute symptoms was usually obtained in 48 hours and one could confidently anticipate that the meatus would be clean and dry in 7 to 10 days. As a prophylactic measure Aurocet (a watery preparation Aluminium Aceto tartrate made by Ward Blenkinsop) was recommended for a further 2 weeks.

The average number of attendances per person was reduced to four and a clinical cure was characterized by the appearance of normal meatal wax. This was usually noted in 4 to 6 weeks after the onset of treatment depending on the rate of recovery of the ceruminous glands. A 90 per cent. cure rate was obtained. The relapse rate was estimated at 5 per cent. and was always associated with an anatomical anomaly of the external auditory meatus which rendered efficient toilet difficult. The remaining 10 per cent. of the cases required admission to hospital for intensive treatment in an air conditioned environment.

Discussion

A characteristic clinical feature of otomycosis is the considerable quantity of epithelial debris which becomes desquamated into the external auditory meatus and extruded to the exterior. This takes the form of an amorphous cheese-like sludge, a compound of a macerated degenerative squames, fungal hyphae and bacteria associated with an offensive smell. When this is removed from the meatus by dry mopping it is commonly observed that the deep meatal skin readily sheds a superficial layer which can be removed by gentle manipulation with a cotton tipped probe. This layer corresponds to a line of cleavage passing through the deep layer of the meatal skin at the dermal/epidermal junction. This tissue may then be subjected to Grocott's differential mycotic stain which shows clearly the fungal hyphae permeating the layers of squames. In fact, the hyphae appear to be actively invasive. The denuded skin is red, raw and extremely tender but free from infection and quickly heals over if toilet and subsequent treatment is adequate. The serial layers of hyphae demonstrated in the "biopsy material" (Fig. 2) are seldom associated with bacteria, suggesting that the fungal infection is primary in many cases and not dependent on the initial devitalization of the tissue by bacterial invasion. This raises the question of the mode of entry of the fungus into the epidermis and the factors which reduce the

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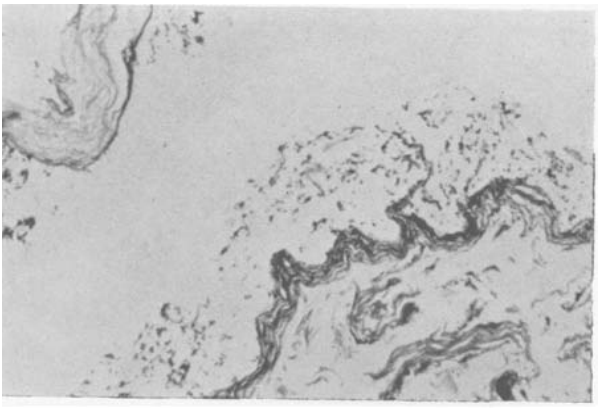


FIG. 2.
This shows packed layers of fungal hyphae within the counterstained epithelium.

viability of the epithelium. The lipid mantle has long been considered as the key factor of this process and its removal by frequent irrigation of the external ear is thought to be the reason why frequent bathing in tropical climates is incriminated as the cause of recurrent infections. In our opinion, swimming in sea or fresh water is not necessarily the mode of entry of infection into the external ear, but the constant irrigation of the outer ear canal whilst swimming and diving repeatedly removes nature's waterproof lining of wax (the lipid mantle). Any factor which subsequently prevents the ready drainage of water from the ear will lead to maceration of the meatal skin and provide an excellent culture medium for the reception of bacterial and fungal spores which are wafted in the atmosphere on water vapour.

In the majority of cases lactophenol blue staining of the aural discharge provided a satisfactory diagnosis of fungal infection. Grocott's staining was used here to obtain an elegant demonstration of fungal invasion of viable meatal epidermis in 71 per cent. of cases examined. In addition, Grocott's stains enabled a detailed inspection of fungal morphology which often provided a specific diagnosis (Fig. 3).

It is our opinion, in common with Smythe (1962), that the lipid mantle is the key factor in determining the onset of otomycosis. In a tropical environment there is a greater tendency for fungal infection to arise as a primary condition.

The high incidence of *Aspergillus niger* and *flavus* occurring alone may also be due to their production of a locally active antibiotic. It is well known that many aspergilli produce antibiotics *in vitro*, but further work is needed to establish that this occurs *in vivo*.

Although it was not possible to obtain clear cut statistical evidence, it was nevertheless accepted by the authors that the widespread use of

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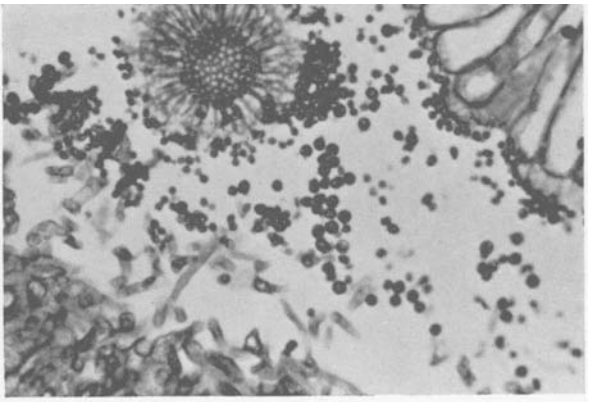


FIG. 3.
This shows a high magnification of one *Aspergillus niger* conidiospore with two layers of Sterigmata. Also there are fungal hyphae invading the epidermis.

broad spectrum antibiotics and steroids combined as a topical application for the ear predisposed to the high incidence of fungal infection in this series.

Summary

Two hundred and twenty-one cases of otorrhoea were investigated which occurred in a European community temporarily residing in a sub-tropical area, in which the incidence of fungal ear infection was considered to be high.

"Aden ear" or "swimmers' ear" is usually associated with frequent sea bathing as this is by far the most popular single recreation and pastime available. Frequent irrigation of the external auditory meatus by warm water, whilst swimming, is considered to be the chief factor in removing cerumen and wax. The subsequent maceration of the meatal skin then provides an excellent culture tube for the germination of fungal spores and growth of pyogenic bacteria.

In the tropics the secretion of sweat, sebaceous and ceruminous glands is considerably increased. This acts as a dilution factor on ear wax and decreases its efficiency as a waterproof lining and makes it more readily removable by irrigation. There is a wide variation in the individual quantity and quality of meatal wax met with during routine aural examinations.

Otomycosis occurs commonly as a primary infection under tropical conditions. Fungal hyphae can be demonstrated as actively invading the epidermis of meatal skin with Grocott's silver stain. We recommend this technique as a reliable means of diagnosis of otomycosis.

Treatment of otomycosis is most satisfactorily provided by Penotrane

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and the use of ear drops is a very inefficient method of medication compared with the use of impregnated ribbon gauze.

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