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Bacterial and mycotic otological infections in Singapore

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SUMMARY

This paper describes a microbiological study of 84 young adult men with clinical otitic infections. Gram-negative aerobic bacilli were frequently isolated from these patients diagnosed as having otitis externa or chronic suppurative otitis media, of which *Pseudomonas* species predominated.

Staphylococcus aureus, *S. epidermidis* and aerobic *Corynebacterium* species (diphtheroids) were also found. About 40% of ear infections were attributed to otomycoses, chiefly from *Aspergillus* species and *Candida parapsilosis*. Antimicrobial susceptibility testing of the bacterial isolates revealed that *Pseudomonas* species were generally resistant to antibiotics commonly employed in general practice: ampicillin, erythromycin, co-trimoxazole, tetracycline and cephaloridine. However, polymyxin B, gentamicin and neomycin were active against some *Pseudomonas* isolates. Other Gram-negative bacilli were also mainly sensitive to gentamicin, neomycin as well as co-trimoxazole. Disc diffusion and minimum inhibitory concentration studies demonstrated good activity of ceftazidime, cefoperazone, tobramycin and carbenicillin against strains of *Pseudomonas* species and other Gram-negative rods. Cefotaxime and cefoxitin were active against Gram-negative bacilli other than *Pseudomonas* species. Beta-lactamase production did not appear to be the main mechanism of resistance in these community-acquired Gram-negative bacillary isolates. The antimicrobial therapy of otological infections is reviewed.

INTRODUCTION

Otological infections are a common cause of morbidity in the community, particularly in a tropical country like Singapore (Meyers & Lawson, 1981). An understanding of the microbiology of these infections and antibiograms lends an insight into a more rational management of the disease and selection of appropriate antimicrobial therapy. A microbiological study was therefore conducted involving 84 young adult men who were clinically diagnosed as having otitis externa or chronic otitis media. Twelve patients had bilateral bacteriologically-proven ear sepsis, while the rest had unilateral involvement. A total of 96 ears were studied, excluding 10 ears of a control group of normal healthy cohorts.

MATERIALS AND METHODS

Patients

All the patients included in the study were defence personnel 18–38 years of age, who were referred to the Ear, Nose and Throat Department of the Medical Classification Centre, Ministry of Defence, Singapore between January to June 1985 for clinical otitis infections. Only those patients who had not received any treatment in the past 1 month, and had clinically obvious ear discharge were studied. All cases were asked about symptoms such as ear ache, discharge, history of previous episodes, and predisposing factors including local manipulation and swimming. The presence of pus, debris and inflammation in the external auditory meatus (EAM) was classified as otitis externa (OE) and a provisional diagnosis of otomycosis was made if masses of mycelia and spores were visible in the EAM. Those with perforation of the tympanic membrane were diagnosed as chronic suppurative otitis media (CSOM). None presented with acute otitis media. Ear culture specimens from the diseased ears were obtained using commercial swabs (Sterilin, Middlesex) for bacterial and fungal culture. Ten per cent potassium hydroxide preparations of smears of ear discharge material were examined microscopically for mycelia, spores or yeast cells. Ear swabs from a group of 10 asymptomatic cohorts without any clinical evidence of otitic infections were additionally taken as controls.

Bacterial and fungal isolation and identification

Ear swabs were inoculated on both blood agar (Oxoid, Basingstoke) and Sabouraud's dextrose agar (Oxoid, Basingstoke), and incubated aerobically at 37 and 30 °C respectively. Gram-negative bacterial isolates were identified with API 20 NE and API 20 E systems (API System S.A., France). Yeast identification was done using API 20 C strips (API System S.A., France). Moulds were identified by colonial and microscopic morphological characteristics, and by the slide culture technique using potato dextrose agar blocks (Riddell, 1950).

Antimicrobial susceptibility testing

This was performed by the disk diffusion method of Kirby and Bauer. Antibiotic disks (Oxoid, Basingstoke) used were ampicillin 10 μg, cephaloridine 30 μg, erythromycin 10 μg, gentamicin 10 μg, neomycin 10 μg, polymyxin B 300 U, tetracycline 30 μg, and trimethoprim-sulphamethoxazole 25 μg. All pseudomonads and other Gram-negative bacterial isolates were also tested against carbenicillin 100 μg, cefoperazone 30 μg, cefotaxime 30 μg, cefoxitin 30 μg, ceftazidime 30 μg, and tobramycin 10 μg (Mast, Liverpool).

Minimum inhibitory concentration (MIC)

MICs were determined by the agar dilution technique on Mueller–Hinton agar (Gibco, Madison) using inocula of about 10^5 c.f.u. Antibiotic powders of known potency (Mast, Liverpool) were used, and doubling antibiotic dilutions from

128 mg/l to 0·25 mg/l were tested against 16 isolates of *Pseudomonas* and 7 other Gram-negative strains. The antibiotics and their recommended breakpoints (in mg/l) were cefotaxime ($\leqslant$ 16), cefoxitin ($\leqslant$ 16), ceftazidime ($\leqslant$ 16), and tobramycin ($\leqslant$ 4).

Beta-lactamase detection

A total of 23 strains of *Pseudomonas* and 8 other Gram-negative isolates were tested for beta-lactamase production by a qualitative, modified acidometric filter paper test (Sng, Yeo & Rajan, 1981).

RESULTS

Clinical features

Of the 84 patients studied, 54 were diagnosed as having OE while the rest had CSOM. Of patients in both categories 14% had bilateral involvement. The commonest presenting complaints in either group were ear ache and discharge. A large proportion of cases volunteered a previous history of similar illness. Local ear manipulation was also a common feature in about two-thirds each of OE and CSOM cases.

Otitis externa

Bilateral involvement was seen in eight cases. Only two cultures were negative. Of 60 ears with positive culture, 56·6% yielded bacterial, 6·7% fungal, and 36·7% both bacterial and fungal isolates. A single organism was found in 45% of positive cultures, two organisms in 53%, and three organisms in 2%. A total of 94 organisms were isolated of which 72% were bacterial and 28% fungal.

Gram-positive organisms constituted 56% of all bacterial isolates, while 44% were Gram-negative bacilli. *Pseudomonas* species formed 70% of the last group, and the remainder were primarily members of Enterobacteriaceae (Table 1).

Of the 47 ears clinically diagnosed as OE of bacterial origin, 13 (27%) yielded positive fungal cultures. Thirteen out of 15 ears presumed to have otomycoses had positive cultures for fungi. Thus, fungal forms were recovered from a significant proportion (43%) of 'positive' ears, *Aspergillus* species and *Candida parapsilosis* being the predominant species.

Otitis media

Thirty patients presented with CSOM, of whom four had bilateral sepsis. Of the 34 ears with positive culture, the majority or 76% yielded a single organism, 15% had two bacterial organisms, while 9% had both bacterial and fungal isolates.

Of the bacteria isolated 70% were Gram-negative bacilli, chiefly *Pseudomonas* species which alone constituted 45%. Only 3 fungal forms were recovered – 2 *Aspergillus* species and 1 *Scedosporium apiospermum* (*Petriellidium boydii*). The latter is a well-documented agent of mycetoma, but has also been reported to be a rare cause of otomycosis (Rippon & Carmichael, 1976).

Controls

Fifteen bacterial isolates were obtained from the 10 control subjects. Eight isolates were diphtheroids, 4 were *Staphylococcus aureus*, while 3 were *S. epidermidis*. No fungi were recovered.

Table 1. *Numbers and percentages of aerobic bacterial and fungal isolates from OE and CSOM cases*

Bacteria	OE		CSOM	
	No.	%	No.	%
Pseudomonas species	21	30·8	18	45·0
P. aeruginosa	17	25·0	16	40·0
P. putida	2	2·9	1	2·5
P. cepacia	2	2·9	—	—
P. fluorescens	—	—	1	2·5
Gram-negative species	9	13·3	10	25·0
Proteus mirabilis	4	5·9	3	7·5
Klebsiella pneumoniae	1	1·5	1	2·5
K. oxytoca	—	—	1	2·5
K. ozaenae	—	—	1	2·5
Citrobacter species	1	1·5	1	2·5
Vibrio alginolyticus	2	2·9	—	—
Achromobacter xylosoxidans	—	—	1	2·5
Enterobacter cloacae	—	—	1	2·5
Providencia species	—	—	1	2·5
Serratia liquefaciens	1	1·5	—	—
Gram-positive species	38	55·9	12	30·0
Staphylococcus aureus	11	16·2	5	12·5
S. epidermidis	14	20·6	3	7·5
Diphtheroids	13	19·1	4	10·0
Total	68	100·0	40	100·0
Fungi				
Aspergillus niger	6	23·1	—	—
A. fumigatus	3	11·5	—	—
Other *Aspergillus* species	9	34·6	2	66·7
Candida parapsilosis	5	19·2	—	—
C. guilliermondii	1	3·8	—	—
Acremonium species	1	3·8	—	—
Fusarium species	1	3·8	—	—
Scedosporium apiospermum	—	—	1	33·3
Total	26	100·0	3	100·0

Direct microscopy

Direct microscopic diagnosis of otomycosis by potassium hydroxide preparations of smears showed a reasonable correlation with fungal culture results. It is therefore a useful preliminary test for the presumptive diagnosis of otomycosis. However, false-negative and false-positive results made up about 17%, hence underlining the need for fungal cultures.

Antibiotic susceptibiliy testing

The antibiograms of all bacterial isolates from patients with OE or CSOM were analysed. Of *Pseudomonas* strains 95 and 82 % were sensitive to polymyxin B and gentamicin respectively. The other standard antibiotics showed poor activity against the *Pseudomonas* species. Other Gram-negative species were generally sensitive to gentamicin, co-trimoxazole, neomycin and ampicillin. Erythromycin was predictably without activity against all Gram-negative isolates. While most of the antibiotics were effective against *S. aureus* strains, a significant proportion

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Table 2. *Antimicrobial susceptibility patterns of 108 bacterial isolates* (%)

Organism	Gentamicin	Polymyxin B	Neomycin	Co-trimoxazole
Pseudomonas species	82	95	26	5
Other Gram-negative bacilli	95	47	68	84
Staphylococcus aureus	100	38	100	88
S. epidermidis	100	94	82	76
Diphtheroids	100	94	94	82
	Tetracycline	Ampicillin	Cephaloridine	Erythromycin
Pseudomonas species	18	0	3	0
Other Gram-negative bacilli	42	68	37	0
Staphylococcus aureus	56	31	94	94
S. epidermidis	82	53	88	94
Diphtheroids	94	59	100	82

were resistant to ampicillin, polymyxin B and tetracycline. The antimicrobial susceptibility patterns of *S. epidermidis* and diptheroids were generally good, with the exception of ampicillin (Table 2).

Susceptibility tests with antimicrobials active against *Pseudomonas* species and other Gram-negative bacteria were analysed. Those found to have highest *in vitro* activity against *Pseudomonas* species were ceftazidime (100%), cefoperazone (97%), tobramycin (91%), and carbenicillin (88%). Other Gram-negative organisms were also generally susceptible to this group of antibiotics.

MIC

Ceftazidime (100%) and tobramycin (94%) were active against *Pseudomonas* isolates, and demonstrated good MIC 50 and MIC 90 values. Other Gram-negative bacilli were sensitive to tobramycin (86%), cefoxitin (71%), ceftazidime (57%), and cefotaxime (57%).

Beta-lactamase studies

Only 13% of *Pseudomonas* strains and 25% of other Gram-negative isolates were beta-lactamase producers despite the high frequency of resistance to beta-lactam antibiotics.

DISCUSSION

Bilateral ear involvement was observed in 14% of both OE and CSOM. The main symptom was ear discharge, while a significant percentage of cases gave a history of previous illness, notably 90% of CSOM patients in whom recurrent infection is a common complication (Nelson, 1982). Two-thirds of OE patients gave a history of ear manipulation with a variety of contaminated devices ranging from cotton buds, hair pins, metallic ear diggers to matchsticks, at weekly or even daily intervals. Many patients volunteered a history of swimming prior to the onset of symptoms. High humidity and temperature, swimming, localized trauma and diminished local tissue resistance are some of the aetiological factors in the multiplex pathogenesis of OE, earning it reputations such as 'hot weather ear',

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'swimmer's ear' and 'Singapore ear' (Wright & Dineen, 1972; Hoadley & Knight, 1975; Meyers & Lawson, 1981; Reid & Porter, 1981).

More than 50% of OE cultures yielded two or more organisms while only 45% grew a single organism. This contrasts with a similar study where 63, 31 and 5% of cultures yielded one, two and three organisms respectively (Cassisi *et al.* 1977). Of ears with positive cultures 43% yielded fungi. This high prevalence of otomycosis compares well with studies in other tropical regions (Than, Naing & Min, 1980) and contrasts with only about 10% in Western reports (Goldstein, 1982). The common occurrence of otomycosis may explain why some OE cases are unresponsive to antibiotic treatment *per se*. Mixed infections may be attributed to self-manipulation, bacterial infection with secondary superimposed mycosis or vice versa, and the increasing use of topical and systemic antibiotics and immunosuppressive corticosteroids. Over half of the bacterial isolates were Gram-positive while 44% were Gram-negative – in contrast to 41 and 59% respectively in the study by Cassisi *et al.* (1977). However, *Pseudomonas* species, in particular *P. aeruginosa*, was the most prevalent organism comprising 70% of Gram-negative isolates (compared with 66% in Cassisi's study). Other Gram-negative genera belonged mainly to the family Enterobacteriaceae. Interestingly, *Vibrio alginolyticus* was isolated from two patients with a history of swimming in sea water, attributed in part to the halophilic nature of the organism. Although *S. aureus*, *S. epidermidis* and diphtheroids such as *Corynebacterium pseudodiphthericum* were recovered, these were also isolated from normal ears. These organisms are considered commensal flora, and are part of the indigenous microbiota of the external auditory canal (Klein, 1981). Despite the uncertainty and controversy of their role in OE and CSOM, some investigators have isolated pure cultures of these microbes from purulent, ear effusions (Feigin *et al.* 1973; Meyerhoff & Giebink, 1982). This together with the high prevalence of local traumatic excoriation suggest that these commensals may assume the role of opportunistic pathogens in the presence of weakened local tissue defences. The commonest fungal species encountered was *Aspergillus* species notably *A. niger* and *A. fumigatus*, which concurs with the findings of other workers (Than, Naing & Min, 1980; Maher *et al.* 1982). *Candida* species other than *C. albicans* were the next most prevalent fungi. Airborne fungi, belonging to the genus *Fusarium*, were also recovered. These have been documented as aetiological agents of otomycosis as well as mycotic keratitis (Bulmer, 1984).

Gram-negative bacillary genera dominated in CSOM, with *Pseudomonas* species being predominant consistent with the spectrum of aetiological agents of CSOM in other reports (Palva & Hallstrom, 1965; Ojala *et al.* 1981; Meyerhoff & Giebink, 1982). *S. aureus*, *S. epidermidis* and diphtheroids were less significant in these CSOM patients. Bacterial CSOM associated with otomycosis accounted for only 9%, comparable to a similar tropical study (Than, Naing & Min, 1980).

Although anaerobic bacteria were not included in this study, they are known to play a role in otitis infections, varying from 0·4 to 41·4% (Jokipii *et al.* 1977; Karma *et al.* 1978; Finegold, 1981; Ojala *et al.* 1981).

S. epidermidis and diphtheroid strains were generally sensitive to most antibiotics commonly employed in general practice. All of these antibiotics except ampicillin and polymyxin B were active against the majority of *S. aureus* strains. The

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majority of *Pseudomonas* isolates were susceptible to polymixin B and gentamicin. Most of the other Gram-negative isolates were also susceptible to the aminoglycosides, gentamicin and neomycin, as well as to co-trimoxazole. Gentamicin or a combination of polymyxin B and neomycin appear to be the most effective conventional antibiotics *in vitro* against these isolates especially *Pseudomonas*. species

First and second generation cephalosporins tested, namely cephaloridine and cefoxitin, were ineffective against *Pseudomonas* species. However, ceftazidime and cefoperazone (third generation cephalosporins), tobramycin and carbenicillin demonstrated good activity against both *Pseudomonas* species and other Gram-negative rods. Cefotaxime and cefoxitin were also useful against some Gram-negative aerobic bacilli other than *Pseudomonas* species.

Beta-lactamase production did not appear to be the main mechanism of antimicrobial resistance in these community-acquired Gram-negative bacillary strains. Plasmids confer on bacteria the ability to produce beta-lactamase, which inactivates beta-lactam antibiotics. These plasmids may carry multiple antibiotic resistance genes, and may be transferred between different bacteria via conjugation (Davies & Smith, 1978).

The selection of appropriate and adequate antimicrobial chemotherapy as part of the overall management, is essential in the prevention of complications of OE and CSOM such as chronicity, recurrence, deafness and intracranial infections.

Topical otic drops containing polymyxin B, colistin or gentamicin are bactericidal to most Gram-negative organisms, notably *Pseudomonas* species and the Enterobacteriaceae. Neomycin and framycetin are broad-spectrum aminoglycosides bactericidal to many Gram-positive and Gram-negative species, particularly *S. aureus* and *Proteus* species. However, most strains of *Pseudomonas* (including 75% of the isolates in this study) are resistant to neomycin (Matz, Lerner & Lanzl, 1981). Ear drop preparations of chloramphenicol have a wide spectrum of activity against Gram-positive and Gram-negative bacteria (except *P. aeruginosa*), and includes anaerobes such as *Bacteroides fragilis* (Palva & Hallstrom, 1965; Fairbanks, 1981).

Anti-fungal drug therapy has a useful role in the treatment of otomycosis, in addition to maintenance of dry ears, avoidance of traumatic manipulation and boric acid ear drops. Several effective anti-mycotic agents with local applications and often with broad-spectrum activity have been recommended: clotrimazole, tolnaftate (Maher *et al.* 1982), amphotericin B, polymyxin B, iodochlorhydroxyquin, nystatin (Lopex & Evens, 1980) and 5-fluorocytosine (Than, Naing & Min, 1980).

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