Mycopathologia **127**: 77–81, 1994.

Survey of mycotic and bacterial keratitis in Sri Lanka

Sirosha A.S. Gonawardena,[1] Kitisiri P. Ranasinghe,[1] Sarath N. Arseculeratne,[1] and C. Reginald Seimon[2] & Libero Ajello[3]
[1]*Department of Microbiology, University of Peradeniya, Peradeniya, Sri Lanka;* [2]*General Hospital, Kandy, Sri Lanka;* [3]*Emory University Eye Center, Ophthalmic Research, Atlanta, Georga, USA*

Received 20 December 1993; accepted in revised form 23 March 1994

Abstract. Over a two-year period (1976–1977 and 1980–1981), 66 cases of bacterial and mycotic cases of keratitis were diagnosed in the Eye Clinic of the General Hospital in Kandy, Sri Lanka. The clinical and microbiologic aspects of these cases are described. Noteworthy was the first known human case caused by *Paecilomyces farinosus*, a geophilic species, commonly encountered as an insect parasite throughout the world. The bacterial and the other fungal etiologic agents isolated and identified were: *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Aspergillus flavus*, *A. niger*, *Fusarium oxysporum*, and *Lasiodiplodia theobromae*. In vitro the fungi showed sensitivity in decreasing order to fluctytosine, nystatin, amphotericin B and econazole. Due to the out-patient status of the patients, their in-vivo response to treatment was not assessable.

Key words: Bacteria, Drug sensitivity, Fungi, Keratitis, *Paecilomyces farinosus*

Introduction

Although infective keratitis, especially of mycotic origin, has been reported from diverse climactic zones, it appears to be more common in subtropical and tropical regions. A global increase in the incidence of this disease has been noted in recent decades, mainly due to the topical use of antibacterial and steroidal preparations [1] which compromise the cornea to opportunistic fungal infections. Due to the non-responsiveness of keratomycosis to these agents, and its spread to cause endophthalmitis, which might require evisceration of the eye, the prompt diagnosis of ocular mycotic infections for appropriate treatment is an urgent need. In addition, these opportunistic infections are preventible and we stress the beneficial results that could be derived from educating the public on preventive measures.

Despite occasional reports from Sri Lanka of keratomycosis caused by unusual fungi [2, 3], the extent of this problem and the causative fungi remain unknown. The magnitude of the problem of corneal ulceration in Sri Lanka can be inferred from the fact that approximately 300 eyes were lost in 1992 in our province alone due to fungal corneal ulcerations (C.R. Seimon, unpublished data). The predominance of initiating injuries from microbially contaminated plant debris and soil, and the fact that this country is primarily agricultural, made such a study warranted. Aggravating factors include the low educational status of farmers and the use by some patients of topical traditional herbal preparations with no proven antifungal action which may aggravate and delay a specific diagnosis and treatment of mycotic keratitis. This study reports on the bacterial and fungal causative agents in 66 Sri Lankan cases of keratitis.

Methods

Patients. Sixty-six consecutive outpatients with corneal ulcerations were examined in 1976–1977 and 1980–1981 in the Eye Clinic of the General Hospital of Kandy. Using sterile scalpels, one of us (C.R.S.) took scrapings from the affected anesthetized corneas. Portions of these were immediately plated for culture and the remainder mounted on slides for microscopy.

Microscopy. The scrapings were placed within a small encirclcd area (for easy location) on microscope slides for 10% KOH mounts and Gram staining. The stock KOH solution and stains were regularly examined for artifacts and contaminant fungi. Fungal filaments were recorded as hyaline or phaeoid, septate or aseptate. Parallel scrapings were cultured on sheep blood and Sabouraud agars at 37 °C (3 days) and at 30 °C (2 weeks) in aerobic incubators. Conventional techniques [4] were used to characterize the bacterial isolates. Fungal cultures from cases with smears negative for fungi were discarded as possible contaminants, although Chin et al. [1] pointed out that negative smears and cultures do not necessarily rule out fungal infection. Cultures of commensal conjunctival bacteria (*Corynebacterium* and *Neisseria* spp. were ignored.

Anti-fungal drug sensitivity tests. These tests were performed as recommended by Casals [5], using tablets of amphotericin B, clotrimazole, econazole, flucytosine, miconazole, and nystatin (gifts from Dr Casals, Rosco, Denmark).

Results

Clinical features. Our patients were all rural adults with predominantly agricultural occupations. The events which probably initiated their ocular infections are presented in Table 1. In the 'no history of trauma' category, traumas may have occurred that were minimal and unnoticed. Jones [6] commented as follows on this matter: 'opportunistic organisms reach the corneal stroma from the preocular tear film through an epithelial defect or as contaminants of injurious material'. Such factors might explain the development of keratomycosis in these patients. A history of topical treatment with traditional, mainly herbal medicaments (predominantly aqueous), was obtained from 32 of the 66 patients, in whom vision had deteriorated despite the treatment. Fourteen had prior 'modern' antibacterial treatment. The duration of the keratitis ranged from 3 days to 6 months, with a modal of 3 to 10 days. Hypopyon was present in 30 patients.

Microscopy. Pus cells were more numerous in smears with pathogenic bacteria, especially *Pseudomonas aeruginosa* and *Streptococcus pneumoniae*, rather than in smears with only fungal pathogens. Smears from 22 cases were positive for filamentous fungi. Of them only 17 were culturally positive. The 5 smear-positive but culture-negative cases were included as cases of keratomycosis in our series [7].

Table 1. Types of traumas involved in 66 cases of ocular keratitis

Nature of trauma	Number of cases
Wood splinter, leaf, seed	20
Soil, stone	6
Graphite	3
Metal fragment	3
Insect bite	1
Ash burn	1
Corrosive fluid	1
Other injury	1
No history of trauma	30

Culture. The genera and species of the bacterial isolates are shown in Table 2. In Table 3 the genera and species of the isolated moulds are presented. The isolation of *Paecilomyces farinosus* from one of our cases of mycotic keratitis is worthy of comment. This species has long been known as a component of the soil's mycobiota and as a widespread polyphagous insect pathogen [8]. In China, this ubiquitous mould is employed in the biological control of the pine caterpillar (*Dendrolimus punctatus* [9]). Previous to our study, *P. farinosus* had not been incriminated as an agent of a human mycosis. In 1989, however, Bruno [10] had described it as a pathogen of farmed Atlantic salmon (*Salmo salar*) causing swim bladder infections. Our isolate was identified as *P. farinosus* by its rapid growth and white, felt-like textured colonies. These assumed a yellowish cast as their elongated conidiophores bore catenulate hyaline, ellipsoidal, smooth, unicellular conidia. Chlamydoconidia were not produced. Its identity was confirmed by Dr A.A. Padhye of the Centers for Disease Control and Prevention, Atlanta, Georgia, USA. The seventeen cases, smear and culture positive for fungi, were negative for bacteria. Among the 22 cases positive for fungi on smear, 5 had bacteria as well. These were identified as *P. aeruginosa* in 2, *S. pneumoniae* in 2, and an unidentified Gram+ coccus in 1. There were no cases with a single bacterial pathogen.

Anti-fungal drug sensitivity. The antifungal drug sensitivity of 6 of the filamentous isolates is shown in Table 4. Except in one of the unidentified isolates, there appeared to be cross resistance between amphotericin B and nystatin [5]. We had no clear evidence of cross resistance to the axole drugs, although 4

Table 2. Genera and species of bacteria isolated from cases of ocular keratitis (n = 66)

Genus/Species	Number
Streptococcus pneumoniae + fungi	2
Pseudomonas aeruginosa + fungi	2
Unidentified B hemolytic coccus	1
Total	5

Table 3. Genera and species of fungi isolated from cases of ocular keratitis (n = 66)

Genus/Species	Number
Aspergillus sp.	1
Aspergillus flavus	1
Aspergillus fumigatus	1
Aspergillus niger	1
Fusarium oxysporum	1
Lasiodiplodea theobromae	1
Paecilomyces farinosus	1
Penicillium sp.	1
Unidentified	14
Total	22 (32%)

isolants gave a 'sensitive', 'intermediate', 'resistant', or an 'intermediate' pattern. One culture was sensitive to econazole but resistant to the other antifungals. The overall sensitivity was as follows: flucytosine – 4 strains, nystatin – 3 strains, amphotericin B – 2 strains, and econazole – 2 strains. With retrospective diagnoses and the outpatient status of our patients, their response to treatment was not assessable.

Discussion

Thygeson & Okumoto [11] in California reported on 15 cases of keratomycosis during the 42 years from 1928–1970, and on 48 cases during 1956–1973. With our 66 cases covering a 2 year period, it might be suggested that this disease has a higher incidence and is therefore of greater importance in poor, tropical countries, which are mainly agricultural. The predominance of preceding ocular traumas by contaminated plant debris, soil or dust, in our cases of keratomycosis agrees with the reported data from other countries. Moreover, it has been our experience that corneal ulceration is commoner during the paddy harvesting season, when corneal traumas from harvesting activities and the winnowing of abrasive, hard-coated paddy seeds might occur. Other factors, such as a low educational status and the use of ill-advised traditional medicaments, might compound the problem. The latter factor has been highlighted, however, in only one report. Puttanna [12] from India reported on five cases of mycotic keratitis that developed after instillation of herbal juices as a part of native treatment. This relationship could be explained, as the author claimed, by the observation that keratomycosis develops on compromised corneas [11]. This is supported by the clinical histories of our patients. Treatment with traditional herbal remedies, either aqueous or oily, could be an additional cause, predisposing to microbial colonization through aggravated damage to the cornea (through irritant plant products) or promotion of fungal growth. This factor would be a hazard in traditional societies. Prevention of keratomycosis should therefore include education of the public on the avoidance of such usage. This type of intervention has indeed resulted in a decrease in the incidence of keratomycosis (C.R. Seimon, unpublished data).

Although a mycotic etiology of keratitis can be inferred from case histories, viz. agricultural occupation and trauma from plant matter or soil, nonresponse to or worsening with long term antibacterial or steroid medication, it is imperative that a confirmatory laboratory diagnosis of a mycotic etiology should be made. This is especially necessary in view of serious complications, such as, endophthalmitis, which could supervene in untreated cases. The acuteness of bacterial and viral keratitis with obvious symptoms and signs prompts earlier treatment. In contrast with the less acute nature and slower progress of most cases of mycotic keratitis, individuals infected by fungi delay seeking medical attention. As a consequence of such procrastination, they present themselves with advanced types of keratomycosis thus making treatment more challenging. The occurrence of greater numbers of pus cells in smears from bacterial keratitis, as we observed, might be a useful criterion for suspecting bacterial keratomycosis. On the other hand, a history of agricultural injury, the use of herbal topical remedies, a paucity of pus cells in corneal smears and a poor response to antibacterial eye drops, apart from the clinical criteria suggested by Kaufman & Wood [13], would lead one to suspect mycotic keratitis. They reported a similar duration of

Table 4. Drug sensitivity of fungi and bacteria isolated from cases of keratitis

Genus/Species	Drug					
	Polyenes		Azoles			
	A	N	E	C	M	F
Aspergillus flavus	0 (R)	9 (R)	8 (R)	9 (R)	5 (R)	36 (S)
Aspergillus fumigatus	11 (I)	20 (S)	13 (I)	13 (I)	11 (R)	33 (S)
Fusarium oxysporum	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	9 (R)
Unidentified	0 (R)	22 (S)	25 (S)	15 (S)	18 (I)	0 (R)
Unidentified	20 (S)	22 (S)	0 (R)	18 (I)	14 (I)	60 (S)
Unidentified	28 (S)	30 (S)	0 (R)	12 (I)	0 (R)	80 (S)
Unidentified	0 (R)	0 (R)	30 (S)	15 (R)	0 (R)	0 (R)
Unidentified	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)

An unidentified filamentous fungus showed no inhibition by any of the drugs tested.
A = amphotericin B; N = Nystatin; E = Econazole; C = Clotrimazole; M = Miconazole; F = Flucytosine.
Numbers represent inhibition zone diameters: S = sensitive; I = intermediate; R = resistant.

the keratitis (10 days–3 weeks) following the initiating event. It is noteworthy that they also stated that 7 of their 15 cases had neither smear nor cultural evidence of fungal infection although, as they emphasized, such negativity did not exclude the possibility of a mycotic infection.

In a series of 9 reports from tropical (India, Nigeria) and temperate countries (USA) in 17 of their 301 cases of keratitis, a fungal etiology was confirmed in 11 (65% compared with 32% in our series). The fungi isolated from our cases, including those that were unidentified, conformed with the data from other studies. *Paecilomyces farinosus*, a pathogen of insects [9] and fish [10], however, had not been previously reported as an etiologic agent of human keratomycosis.

Keratitis, caused by *Fusarium* spp., has been reported to show a variable response to antifungal treatment. Newmark, Ellison & Kaufman [14] reported cure with pimaricin (natamycin) which was superior in effectiveness to nystatin and amphotericin B. In contrast Wood & Williford [15] achieved cure with 0.15% amphotericin B. Flynn [16], however, found a poor response in *F. oxysporum* (as *F. conglutinans*, a synonym) keratitis to amphotericin B and nystatin while cycloheximide proved curative. Our strain of *F. oxysporum* was resistant to amphotericin B and nystatin. Sensitivity to pimaricin was not tested.

That flucytosine had the highest number of sensitive strains contrasts with reports cited by Iwata [17] on the 'considerable number' of cases of primary resistance and the emergence of secondary resistance during therapy with this agent. The occurrence of resistance to the agents tested in our series of cases and reports of resistance to these and other antifungal agents might suggest the value of combined antifungal therapy [17].

With bacteria, the pattern of our isolates, predominantly *S. pneumoniae* (which Thygeson & Okymoto [11], regarded as the principal corneal bacterial pathogen) and *P. aeruginosa*, also conformed to that reported by other authors. The haemolysin of *S. pneumoniae* has been recognized as the pathogenetic factor [16]. The occurrence of *P. aeruginosa* reflects Jones's comment [6]: 'The only exotoxin which has been linked to the pathogenesis of corneal suppuration is *P. aeruginosa* exotoxin, which inhibits cellular protein synthesis, is toxic for macrophages in-vitro and impairs phagocytosis'.

Conclusion

Sixty-six cases of keratitis in outpatients were studied during a 2-year period. The clinical histories, an agricultural status for most patients, recollection of a preceeding trauma from vegetable matter and soil and the patterns of causative fungi and bacteria, were similar to those reported from other tropical (India, Nigeria) as well as temperate (USA) countries viz., *P. aeruginosa*, *S. penumoniae*, among the bacteria and *A. flavus*, *A. fumigatus* and *A. niger* and other unidentified species of aspergilli, *L. theobromae*, and *F. oxysporum* and the *Penicillium* spp. among the fungi. We report the first instance of *P. farinosus* as a pathogen in human keratomycosis. Surprisingly, no yeast-like fungi were encountered. The filamentous fungi showed sensitivity in decreasing order to flucytosine, nystatin, amphotericin B, and econazole.

Acknowledgements

We thank Dr J.B. Casals (Rosco, Denmark) for his gift of the antifungal drug tablets and instructions for their use, and Mr. L.V. Weliange for technical assistance.

References

1. Chin GN, Hundiuk RA, Kwasny GP, Schultz GP. Keratomycosis in Wisconsin. Am J Ophthalmol 1975; 79: 121–25.
2. Nityananda K, Sivasubramaniam P, Ajello L. Mycotic keratitis caused by *Curvularia lunata*. Sabouraudia 1962; 2: 35–9.
3. Nityananda K, Sivasubramaniam P, Ajello L. A case of mycotic keratitis caused by *Curvularia geniculata*. Arch Ophthalmol 1964; 71: 456–8.
4. Cowan ST, Steel KJ. Manual for the identification of medical bacteria, 2nd ed. Cambridge: Cambridge University Press, 1974.
5. Casals JB. Tablet sensitivity testing of pathogenic fungi. J Clin Pathol 1979; 32: 719–22.
6. Jones DB. Pathogenesis of bacterial and fungal keratitis. Trans Ophthalmol Soc UK 1978; 98: 367–71.
7. Fedukowic HB, Stenson S. External infections of the eye. Norwalk: Appleton, Century and Crofts, 1985: 61.
8. Samson RA. *Paecilomyces* and some allied hyphomycetes. Studies in mycology, Vol. 6. Baarn: Centraalbureau voor Schimmelcultures, 1974.
9. Ferron P, Fargues J, Riba G. Fungi as microbial insecticides against pests. In: Arora DK, Ajello L, Mukerji KG, eds. Handbook of applied mycology, Vol. 2: Humans, animals and insects. New York/Basel/Hong Kong: Marcel Dekker, 1991: 665–706.
10. Bruno DW. Observations on a swim bladder fungal infection of farmed Atlantic salmon, *Salmo salar* L. Bull Assoc Eur Fish Pathol 1989; 9: 7–8.
11. Thygeson P, Okumoto M. Keratomycosis: A preventable disease. Trans Am Acad Ophthalmol Otolaryngol 1974; 8: 433–9.
12. Puttanna ST. In Trans Asia-Pacific Acad Ophthalmol 1964; 2: 298–305, cited in Ophthalmic Literature, 1965.
13. Kaufman E, Wood RM. Mycotic keratitis. Am J Ophthalmol 1965; 59: 993–1000.
14. Newmark E, Ellison AC, Kaufman HE. Pimaricin therapy of *Cephalosporium* and *Fusarium* keratitis. Am J Ophthalmol 1970; 69: 458–66.
15. Wood TO, Williford W. Treatment of keratitis with amphotericin B 0.15%. Am J Ophthalmol 1976; 75: 847–49.
16. Flynn JR. *Fusarium* keratitis treated with cycloheximide. Am J Ophthalmol 1964; 58: 637–41.
17. Iwata K. Drug resistance in human pathogenic fungi. Eur J Epidemiol 1992; 8: 407–21.

Address for corresondence: Professor S.N. Arseculeratne, Department of Microbiology, Faculty of Medicine, University of Peradeniya, Peradenya, Sri Lanka
Phone: 94-8-88130; Fax: 94-8-32572