SCIENTIFIC REPORT

Prevalence and spectrum of bacterial co-infection during fungal keratitis

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Aims: To estimate the propensity of keratomycosis for parallel or secondary bacterial infection and to explore affinities among fungal and bacterial co-isolates.

Methods: A retrospective review of laboratory records over 24 years yielded 152 episodes of culture positive fungal keratitis. After collating 65 corneal specimens having bacterial co-isolates, polymicrobial co-infection was defined as detection of concordant bacteria on smear and culture or on two or more different media.

Results: 30 (20%) keratomycoses met laboratory criteria for polymicrobial infection. The risk of bacterial co-infection was 3.2 (95% confidence interval, 1.7 to 5.8) times greater with yeast keratitis than with filamentous fungal keratitis.

Conclusions: Bacterial co-infection occasionally complicates fungal keratitis, particularly candidiasis.

Fungal keratitis is a sight threatening infection caused by yeasts or moulds. Concomitant bacterial infection is an added diagnostic and therapeutic dilemma. Building on a previous report examining polymicrobial keratitis from our laboratory,¹ we undertook this retrospective laboratory study to examine the prevalence of bacterial co-infection during fungal keratitis.

MATERIALS AND METHODS

We reviewed request forms and laboratory records regarding corneal scrapings and biopsies collected between January 1981 and December 2004. Corneal specimens were routinely smeared onto glass slides for staining and directly inoculated onto culture media, including blood and chocolate agar plates incubated at 35°C and Sabouraud agar incubated at 25°C.² The laboratory criterion for fungal keratitis was fungal growth on at least one culture medium. Fungal elements on smear without confirmatory fungal culture were not considered sufficient for this study.

Fungi were recovered from 152 corneal specimens obtained from 146 eyes. Six eyes that had the same filamentous fungus isolated on repeat corneal sampling more than 3 weeks after the first specimen were included to capture sequential as well as simultaneous bacterial co-isolates. Bacterial co-infection was defined as growth on at least one

medium of bacterial organisms seen on stained smears of corneal scrapings or as growth of the same species on two or more media. After subculture and identification of bacterial co-isolates, the principal corneal isolate for eyes with multiple bacterial species was defined by the results of stained corneal smears and then by the following ordered sequence: Gram negative rods, Gram positive cocci other than *Staphylococcus epidermidis* or *Micrococcus* species, Gram positive rods other than *Propionibacterium* species and *Corynebacterium* sp, *S epidermidis* or *Micrococcus* species, then propionibacteria and coryneforms.³ For eyes with more than one species within these groups, the principal isolate was chosen by the greatest amount of growth.

Analyses were performed using statistical software (Intercooled Stata version 9, StataCorp, College Station, TX, USA). Risk ratios with confidence intervals compared the relative frequency of bacterial infection between yeast and filamentous fungal keratitis. Logistic regression explored potential risk factors associated with mixed keratitis compared to fungal keratitis lacking bacterial co-infection, adjusted for fungal phylum. Associations among smear and culture results were compared by χ^2 tests.

RESULTS

Sixty five (42.8%) of 152 episodes of culture positive fungal keratitis yielded bacterial growth; 30 (19.7%) met laboratory criteria for bacterial co-infection (table 1). Twenty three had similar bacteria seen on smears and recovered on at least two culture media, while three had positive smears with one positive culture and four had bacteria found on multiple media but not on smears.

Bacterial co-infection occurred 3.16 (95% confidence interval 1.72 to 5.78) times more often with yeast keratitis than with filamentous fungal keratitis ($p = 0.0002$). The risk did not significantly differ between moniliaceous (hyaline) and dematiaceous fungal keratitis ($p = 0.16$). After adjusting for fungal type, no demographic characteristic was clearly associated with co-infection (table 2).

The sensitivity of detecting fungal elements, regardless of bacterial co-infection, using microscopic examination of corneal smears by any stain was 57% (95% confidence interval, 48% to 66%), including 56% by staining with acridine orange and 68% with calcofluor white. Among co-infected eyes, bacteria were detected by any stain in 47%

Table 1 Bacterial co-isolation and co-infection in fungal keratitis

Fungal corneal isolate	Eyes (n = 152)	Bacterial growth (n = 65)	Bacterial co-infection (n = 30)
Yeasts	33	20 (60.6%)	14 (42.4%)
Moniliaceous filamentous fungi*	78	26 (33.3%)	8 (10.3%)
Dematiaceous filamentous fungi	41	19 (46.3%)	8 (19.5%)

*Includes four non-specified moulds as well as one moniliaceous hyphomycete co-isolated with a pigmented mould.

Table 2 Clinical characteristics of fungal keratitis according to bacterial co-infection

Characteristic	Fungal keratitis without co-infection (n = 122)	Fungal keratitis with bacterial co-infection (n = 30)	Odds ratio (95% confidence interval)*	p Value
Age, years (mean (SD))	48 (19)	56 (17)	1.02 (0.99 to 1.04)	0.19
Females (%)	40 (33%)	17 (57%)	2.20 (0.94 to 5.17)	0.07
Previous antibiotic (%)	37 (30%)	11 (37%)	1.24 (0.52 to 3.00)	0.63
Season (months)				
Spring (March-May)	29 (24%)	6 (20%)	1.00 (referent)	–
Summer (June-August)	36 (30%)	9 (30%)	1.23 (0.37 to 4.08)	0.74
Autumn (September-November)	44 (36%)	8 (27%)	1.31 (0.38 to 4.54)	0.67
Winter (December-February)	13 (11%)	7 (23%)	3.14 (0.81 to 12.2)	0.10

*Adjusted for fungal type.

(95% confidence interval, 34% to 61%), including 47% by acridine orange and 50% by Gram staining (table 3).

Irrespective of bacterial co-infection, fungi were recovered on 80% of blood agar plates, 77% of chocolate agar plates, and 74% of Sabouraud agar plates or slants. Cumulatively, these three primary isolation media grew 95% of all fungal isolates. Two patients with polyfungal keratitis were encountered: one with two *Candida* species and one with two filamentous fungi (*Aspergillus* and *Curvularia*). Among co-infected eyes, bacterial co-isolates were found on 52% of inoculated blood agar plates, 46% of chocolate agar plates, and 67% of thiol or thioglycolate broth tubes (table 4). Cumulatively, these three media allowed recovery of 92% of all bacterial co-isolates, while blood agar and thioglycolate together detected 84%.

The bacterial spectrum associated with fungal keratitis comprised 65 principal and 24 additional bacterial coisolates (table 5). Of these 89 bacterial isolates, 45 (51%) were Gram positive cocci (29 coagulase negative staphylococci, three *Staphylococcus aureus*, seven viridans streptococci, two enterococci, one *Streptococcus pneumoniae*, one *Micrococcus* sp, one *Aerococcus* sp, and one *Peptostreptococcus* sp), 29 (33%) were Gram positive rods (14 propionibacteria, 10 corynebacteria, two *Eubacterium aerofaciens*, one *Bacillus* sp, and two not speciated), and 15 (17%) were Gram negative rods (four *Pseudomonas* sp, one *Alcaligenes xylosoxidans*, one *Flavobacterium*

sp, three *Klebsiella pneumoniae*, one *Citrobacter* sp, one *Proteus* sp, one *Moraxella* sp, one *Fusobacterium* sp, one *Capnocytophaga* sp, and one not speciated). Gram positive cocci were equivalently associated with yeasts and filamentous fungi ($p = 0.88$), while Gram negative rods were more often associated with yeasts ($p = 0.0002$) and Gram positive rods with filamentous fungi ($p = 0.002$).

DISCUSSION

Twenty per cent of eyes with culture positive fungal keratitis in this study had laboratory defined bacterial co-infection. Others have recovered bacterial co-pathogens in 5% or less,⁴⁻⁶ but recent series from India found bacteria in 5% to 25% of keratomycoses.⁶⁻¹³ Other laboratories in Asia and South America have isolated bacteria in approximately 30% to 60% of corneal specimens during fungal keratitis.¹⁴⁻¹⁶ These diverse prevalence estimates may indicate non-conformity in distinguishing microbial co-isolation and dual infection but could be due to differences in risk factors, climates, and access to care.

The diagnosis of fungal keratitis and the recognition of mixed infection are guided by microscopic examination and culture isolation. Because bacteria in the tear film may be carried by corneal scrapings and be coincidentally isolated, laboratory standards are needed to help confirm

Table 3 Sensitivity of corneal smears for culture-positive keratomycosis

Stain	Fungal keratitis without co-infection		Fungal keratitis with bacterial co-infection			
	Examined	Fungal elements	Examined	Fungal elements only	Fungi and bacteria	Bacteria only
Gram	54	32 (59%)	52	10 (19%)	10 (19%)	16 (31%)
Acridine orange	31	19 (61%)	17	4 (24%)	4 (24%)	4 (24%)
Calcofluor white	21	16 (76%)	7	3 (43%)	0	0
Any stain	71	48 (68%)	55	14 (25%)	10 (18%)	16 (29%)

Table 4 Culture media used for keratomycosis

Medium	Fungal keratitis without co-infection			Fungal keratitis with bacterial co-infection				
	Inoculated	Fungal growth	Cumulative proportion with fungal growth	Inoculated	Fungal growth	Cumulative proportion of fungal growth	Bacterial growth	Cumulative proportion with bacterial growth
Blood agar plate	84	75 (89%)	0.86	63	42 (67%)	0.67	33 (52%)	0.52
Chocolate agar plate	85	73 (86%)	0.95	61	39 (64%)	0.83	28 (46%)	0.62
Sabouraud agar plate	42	33 (79%)	0.99	28	19 (68%)	0.90	0	0.62
Brain-heart infusion	32	27 (84%)	1.00	20	15 (75%)	0.98	0	0.62
Thiol or thioglycolate broth	65	33 (51%)	1.00	42	6 (14%)	1.00	28 (67%)	0.92
Schaedler or <i>Brucella</i> agar plate	23	14 (61%)	1.00	22	5 (23%)	1.00	16 (73%)	1.00

Table 5 Joint associations of 65 principal and 24 additional bacterial co-isolates during polymicrobial keratomycosis

Fungal corneal isolate	Bacterial co-isolates	Gram positive cocci	Gram positive rods	Gram negative rods
<i>Candida</i> sp	28	15	3	10
Other yeast	1	0	0	1
<i>Fusarium</i> sp	18	7	10	1
<i>Aspergillus</i> sp	9	4	3	2
<i>Curvularia</i> sp	9	5	4	0
Other filamentous fungi	24	14	9	1

polymicrobial keratitis. Fluorescent microscopy using calcofluor white¹⁷ or acridine orange¹⁸ disclosed fungal elements in the majority of our keratomycosis specimens, and Gram staining provided adjunctive help in discerning fungi and bacteria.^{11 13 19 20} An agar plate was effective for microbial recovery,²¹ but multiple primary culture media were needed to isolate all micro-organisms from corneal scrapings of co-infected eyes. No single staining method or culture medium appeared substantially more effective for detecting fungi and bacteria.

Staphylococci are relatively prevalent during fungal keratitis,²² comprising half of our patients' bacterial co-isolates. Case reports have described less common bacterial species during fungal keratitis.^{23 24} We noticed that some bacterial groups selectively paired with particular fungal infections suggesting that co-pathogens may form a mutual alliance.^{25–27} Polymicrobial synergism can influence the severity of an intermingled infection,^{28–30} but the interactions between fungi and bacteria that foster co-infection need to be better explained.^{31 32}

The pathogenesis of mixed infection may depend on the source and sequence of microbial exposure. In this study, bacterial co-infection occurred three times more often with yeasts than with filamentous fungi, implying a communal, possibly indigenous, reservoir for *Candida* and coagulase negative staphylococci. *Candida albicans* forms biofilm³³ that allows bacteria to adhere and to survive.³⁴ Administering bacterial contaminants to the eye, either simultaneously with or subsequent to a mycotic pathogen, worsens experimental fungal keratitis.³⁵ Superinfection by normal bacterial flora during early keratomycosis is a possible pathway leading to polymicrobial corneal infection.

Estimating the prevalence and attributes of dual bacterial and fungal keratitis was limited by our study's retrospective, laboratory based design. Full laboratory assessment was not always feasible. Valid bacterial isolates may have been missed by insufficient corneal sampling or antibiotic carryover while micro-organisms of the ocular surface may have been inappropriately included. Under-reporting of preceding corneal injury, misclassification of recent antibiotic use, omission of ocular surface disease³⁶ and previous corneal surgery,^{37 38} and other informational shortcomings weakened our ability to verify an ecological risk factor for bacterial co-infection. As the diagnosis of co-infection was limited to laboratory criteria, the choice and effect of antimicrobial therapy were not evaluated. Our findings may not be generalisable to populations having the greatest burden of fungal keratitis.

Future studies of polymicrobial keratomycosis should standardise diagnostic ascertainment and incorporate therapeutic response and outcome, particularly the selection and effects of combined antifungal and antibacterial therapy. Ongoing advances in ocular microbiology offer opportunities to improve clinical decision making for complex microbial keratitis.

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