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, 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. 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 order of 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. 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 $\chi^2$ 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% of cases.

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

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

*Includes four non-specified moulds as well as one moniliaceous hyphomycete co-isolated with a pigmented mould.
Two Gram positive rods (14 propionibacteria, 10 corynebacteria, and one Aerococcus 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 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 (33%) were Gram negative rods, 15 (17%) were Gram negative cocci (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 coccIs 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% 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

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

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

*Adjusted for fungal type.

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**Table 3** Sensitivity of corneal smears for culture-positive keratomycosis

<table>
<thead>
<tr>
<th>Stain</th>
<th>Examinable</th>
<th>Fungal elements</th>
<th>Examinable</th>
<th>Fungal elements only</th>
<th>Fungi and bacteria</th>
<th>Bacteria only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram</td>
<td>54</td>
<td>32 (59%)</td>
<td>52</td>
<td>10 (19%)</td>
<td>10 (19%)</td>
<td>16 (31%)</td>
</tr>
<tr>
<td>Acridine orange</td>
<td>31</td>
<td>19 (61%)</td>
<td>17</td>
<td>4 (24%)</td>
<td>4 (24%)</td>
<td>4 (24%)</td>
</tr>
<tr>
<td>Calcofluor white</td>
<td>21</td>
<td>16 (76%)</td>
<td>7</td>
<td>3 (43%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Any stain</td>
<td>71</td>
<td>48 (68%)</td>
<td>55</td>
<td>14 (25%)</td>
<td>10 (18%)</td>
<td>16 (29%)</td>
</tr>
</tbody>
</table>

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**Table 4** Culture media used for keratomycosis

<table>
<thead>
<tr>
<th>Medium</th>
<th>Inoculated</th>
<th>Fungal growth</th>
<th>Cumulative proportion with fungal growth</th>
<th>Inoculated</th>
<th>Fungal growth</th>
<th>Cumulative proportion of fungal growth</th>
<th>Bacterial growth</th>
<th>Cumulative proportion with bacterial growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood agar plate</td>
<td>84</td>
<td>75 (89%)</td>
<td>0.86</td>
<td>63</td>
<td>42 (67%)</td>
<td>0.67</td>
<td>33 (52%)</td>
<td>0.52</td>
</tr>
<tr>
<td>Chocolate agar plate</td>
<td>85</td>
<td>73 (86%)</td>
<td>0.95</td>
<td>61</td>
<td>39 (64%)</td>
<td>0.83</td>
<td>28 (46%)</td>
<td>0.62</td>
</tr>
<tr>
<td>Sabouraud agar plate</td>
<td>42</td>
<td>33 (79%)</td>
<td>0.99</td>
<td>28</td>
<td>19 (68%)</td>
<td>0.90</td>
<td>0</td>
<td>0.62</td>
</tr>
<tr>
<td>Brain-heart infusion</td>
<td>32</td>
<td>27 (84%)</td>
<td>1.00</td>
<td>20</td>
<td>15 (75%)</td>
<td>0.98</td>
<td>0</td>
<td>0.62</td>
</tr>
<tr>
<td>Thiol or thioglycolate</td>
<td>65</td>
<td>33 (51%)</td>
<td>1.00</td>
<td>42</td>
<td>6 (14%)</td>
<td>1.00</td>
<td>28 (67%)</td>
<td>0.92</td>
</tr>
<tr>
<td>Broth</td>
<td>23</td>
<td>14 (61%)</td>
<td>1.00</td>
<td>22</td>
<td>5 (23%)</td>
<td>1.00</td>
<td>16 (73%)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

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polymermic keratitis. Fluorescent microscopy using calco-
fluor white$^{17}$ or acidine orange$^{18}$ 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 kera-
titis,$^{25}$ comprising half of our patients’ bacterial co-isolates.
Case reports have described less common bacterial species
during fungal keratitis.$^{26}$ 27 We noticed that some bacterial
groups selectively paired with particular fungal infections
suggesting that co-pathogens may form a mutual alliance.$^{25}$
Polymicrobial synergism can influence the severity of an
intermingled infection,$^{26}$ 27 but the interactions between fungi
and bacteria that foster co-infection need to be better
explained.$^{25}$ 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 biofilm$^{33}$ that
allows bacteria to adhere and to survive.$^{44}$ 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
polymermic 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 cor-
neal injury, misclassification of recent antibiotic use, oми-
sion of ocular surface disease$^{44}$ and previous corneal surgery,$^{47}$ 48 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 therapeu-
tic 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.

| **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** |
| Candida sp                  | 28                | 15                | 3                 | 10                |
| Other yeast                 | 1                 | 0                 | 0                 | 1                 |
| Fusarium sp                 | 18                | 7                 | 1                 | 10                |
| Aspergillus sp              | 9                 | 4                 | 3                 | 2                 |
| Curvularia sp               | 9                 | 5                 | 4                 | 0                 |
| Other filamentous fungi     | 24                | 14                | 9                 | 1                 |

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