Inhibitory effects of antimicrobial agents against *Fusarium* species

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Abstract

We investigated the inhibitory effects of antibacterial, biocidal, and antifungal agents against *Fusarium* spp. Seven *Fusarium* spp: four *F. falciforme* (*Fusarium solani* species complex), one *Fusarium* spp, one *Fusarium* spp. (*Fusarium incarnatum-equiseti* species complex), and one *F. napiforme* (*Gibberella fujikuroi* species complex), isolated from eyes with fungal keratitis were used in this study. Their susceptibility to antibacterial agents: flomoxef, imipenem, gatifloxacin, levofloxacin, moxifloxacin, gentamicin, tobramycin, and Tobracin® (contained 3,000 μg/ml of tobramycin and 25 μg/ml of benzalkonium chloride (BAK), a biocidal agent: BAK, and antifungal agents: amphotericin B, pimaricin (natamycin), fluconazole, itraconazole, miconazole, voriconazole, and micafungin, was determined by broth microdilution tests. The half-maximal inhibitory concentration (IC₅₀), 100% inhibitory concentration (IC₀₀₀), and minimum inhibitory concentration (MIC) against the *Fusarium* isolates were determined. BAK had the highest activity against the *Fusarium* spp. except for the antifungal agents. Three fluoroquinolones and two aminoglycosides had inhibitory effects against the *Fusarium* spp. at relatively high concentrations. Tobracin® had a higher inhibitory effect against *Fusarium* spp. than tobramycin alone. Amphotericin B had the highest inhibitory effect against the *Fusarium* spp, although it had different degrees of activity against each isolate. Our findings showed that fluoroquinolones, aminoglycosides, and BAK had some degree of inhibitory...
effect against the seven *Fusarium* isolates, although these agents had considerably lower effect than amphotericin B. However, the inhibitory effects of amphotericin B against the *Fusarium* spp. varied for the different isolates. Further studies for more effective medications against *Fusarium*, such as different combinations of antibacterial, biocidal, and antifungal agents are needed.

**Key words:** *Fusarium*, antibacterial agent, biocidal agent, antifungal agent, inhibitory effect.

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**Introduction**

Fungal keratitis is a serious ocular disease that can lead to a severe decrease of vision, ocular phthisis, and enucleation. Therefore, early diagnosis and appropriate treatment with effective antifungal drugs are important for patients who have fungal keratitis. New antifungal agents, such as liposomal-amphotericin B, micafungin, caspofungin, and voriconazole, have been developed. Although these offer more choices for treating fungal keratitis, the best drug to treat a specific *Fusarium* spp. has still not been determined. In addition, there are some problems in using antifungal agents, for example, low intraocular penetration by topical and systemic administration and low activity against filamentous isolates. Therefore, many patients still have poor outcomes.

The first choice for treating fungal keratitis would be antifungal drugs. However, it has been shown that treatment with antibacterial agents alone, for example, topical gentamicin, tobramycin, ceftazolin, mexiteloxacin, gatifloxacin, levofloxacin, and ofloxacin, and intravenous tobramycin and ceftazolin, without any antifungal agents has been effective against selected cases of fungal keratitis [1–7]. There are also in vitro studies suggesting that antibacterial agents have some activity against fungi although limited. Thus, Chodosh et al. found that tobramycin completely inhibited the growth of *F. oxysporum* at a concentration of 450 μg/ml [2]. Ozdek et al. found that the commercial topical preparations of both gatifloxacin and mexiteloxacin had antifungal activity against *Candida* spp. in vitro [8]. Day et al. reported that *Fusarium* spp. isolates were susceptible to chloramphenicol, mexiteloxacin, tobramycin, and benzalkonium chloride (BAK), and *Aspergillus flavus* isolates to chloramphenicol and BAK [9].

It is important for pathogens to be identified accurately to the species level not only for diagnosis and therapy but also for future studies. However, in most of the earlier studies, the pathogen was not identified to the species level [3–9].

The purpose of this study was to investigate the susceptibility of clinical isolates of *Fusarium* to antibacterial, biocidal, and antifungal agents. All of the *Fusarium* spp. were identified to the species complex or species level in the current study.

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**Materials and methods**

**Clinical samples and identification of moulds**

Clinical specimens (corneal swabs) were obtained from patients with keratitis. The collected samples were plated on Sabouraud dextrose agar (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan), incubated at 30°C under controlled humidity and examined daily for up to four weeks. Identification of the moulds was based on the microscopic findings, the morphological characteristics of the colonies in culture, and the DNA sequencing of a partial elongation factor (EF) 1-α gene as described in a previous study [10].

**In vitro susceptibility testing**

Eight antibacterial agents: flomoxef, imipenem, gatifloxacin, levofloxacin, mexiteloxacin, tobramycin, gentamicin, and Tobracin® and seven antifungal agents: amphotericin B, mexiteloxacin, pimaricin (also called natamycin), fluconazole, itraconazole, miconazole, voriconazole, and micafungin, were studied. In addition, one biocidal agent: BAK, was studied. The Tobracin® (Nitto Medic Co., Ltd., Toyama, Japan) contained 3,000 μg/ml of tobramycin and 25 μg/ml of BAK. The other antimicrobials did not contain any preservatives including BAK.

Each conidial suspension was prepared with RPMI-1640 and adjusted the concentration to 2.5 × 10⁸ cells/ml by use of a hemocytometer (Neubauer chamber). Stock solutions of the antibacterial agents and biocidal agent were prepared in RPMI 1640 medium (Sigma-Aldrich, St. Louis, MO, USA) which is a standard medium for fungus because *Fusarium* spp. were the main organisms in this study. Stock solution of pimaricin was prepared in methanol. Further serial dilutions in RPMI 1640 medium were performed in rounded-bottomed 96-well plates. Flomoxef and imipenem were tested at concentrations from 9.766 to 5,000 μg/ml. The levofloxacin and mexiteloxacin concentrations tested ranged from 4.883 to 2,500 μg/ml. Tobramycin and gentamicin concentrations tested ranged from 5.859 to 3,000 μg/ml, and gatifloxacin and Tobracin® concentrations ranged from 2.930 to 1,500 μg/ml. The pimaricin and BAK concentrations tested ranged from 0.078 to 40 and...
from 1 to 512 μg/ml, respectively. Prior to the susceptibility tests, each antibiotic agent was tested against a standard *Staphylococcus aureus* strain to ensure that the MIC was within the published range. The tests against other antifungal agents, amphotericin B, fluconazole, itraconazole, miconazole, voriconazole and micafungin, were performed by the standard M38-A2 broth microdilution method [11] with some modifications using the Dry Plate Eiken (Eiken Chemicals, Tokyo, Japan). The plates were incubated at 35°C for 48 hours. Tests on each fungal culture were repeated twice on different days by two masked investigators to reduce the effect of random variations in the test results.

The susceptibility of the *Fusarium* spp. was graded by an inhibitory concentration of 100% (IC₁₀₀), IC₅₀, minimum inhibitory concentration (MIC), and minimum effective concentration (MEC). The IC₁₀₀ was defined as the lowest concentration of an agent that completely inhibited the growth of *Fusarium*. IC₅₀ was defined as the lowest concentration of an agent that inhibited approximately 50% of the drug-free growth control. The MIC was defined as the lowest concentration of an agent that led to abnormal growth, such as leading to small, rounded, compact hyphal forms of the fungus. The susceptibility of the *Fusarium* spp. to antibacterial agents, BAK, and pimaricin were graded by the IC₁₀₀ and IC₅₀ values because no standards to estimate inhibitory effects against fungus of these agents existed. Both the IC₁₀₀ and IC₅₀ values of these agents were measured in reference to the concentration used clinically or the concentration of commercial ophthalmic solutions. Susceptibilities to amphotericin B, fluconazole, itraconazole, miconazole, and voriconazole were evaluated by the MIC. Susceptibility to micafungin was evaluated by the MEC. The quality of the measurement was confirmed by examining quality control isolates, for example, *C. parapsilosis* ATCC 22019, *C. krusei* ATCC6285, *A. fumigatus* ATCC 204305, and *A. flavus* ATCC 204304. All quality control readings were within the recommended limits suggested in the standard CLSI procedures.

**Statistical analysis**

Statistical analyses were performed with the Fisher’s exact test. The level of statistical significance was set at a *P* < 0.05.

**Results**

The demographics of the patients are summarized in Table 1. Seven strains of *Fusarium* spp. were isolated from the seven patients. Four isolates were identified as *Fusarium falciforme* (*Fusarium solani* species complex (FSSC)), one isolate as *Fusarium* spp. (FSSC), one isolate as *Fusarium* spp. *Fusarium incarnatum-equiseti* species complex (FESC), and one isolate as *Fusarium napiforme* *Gibberella fujikuroi* species complex (GFSC).

The susceptibilities of these *Fusarium* isolates to each antibacterial and biocidal agents are shown in Table 2. BAK had the lowest IC₅₀ and IC₁₀₀ for all of the *Fusarium* spp. The IC₅₀ for BAK was 4 to 32 μg/ml, and the IC₁₀₀ was 8 to 256 μg/ml. Some of the *Fusarium* isolates were also susceptible to the three fluoroquinolones, and especially to the two aminoglycosides and Tobracin® but only at relatively high concentrations. The IC₅₀ for gatifloxacin, levofloxacin, and moxifloxacin were 750 μg/ml for two isolates, 312.5 to 1,250 μg/ml for six isolates, and 156.25 to 1,250 μg/ml for six isolates, respectively. Only moxifloxacin among the three fluoroquinolones had an IC₁₀₀ (= 2,500) but only for two isolates. The IC₅₀ for tobramycin, gentamicin, and Tobracin® were 187.5 to 375 μg/ml, 93.75 to 187.5 μg/ml, and 93.75 to 187.5 μg/ml, and the IC₁₀₀ was 375 to 3,000 μg/ml except for two isolates, 187.5 to 1,500 μg/ml except for two isolates, and 187.5 to 750 μg/ml, respectively. Neither imipenem nor flomoxef had any detectable activity against the *Fusarium* spp. at concentrations of ≤5,000 μg/ml.

The susceptibility of the *Fusarium* isolates to each antifungal agent is shown in Table 3. Amphotericin B, pimaricin, miconazole, and voriconazole had higher inhibitory effects against the *Fusarium* isolates growth. Amphotericin B had the highest inhibitory effect against the *Fusarium* spp. among all of the antimicrobial agents, although the MIC varied from 1 to 16 μg/ml except for one isolate. The IC₅₀ for pimaricin was 2.5 to 5 μg/ml, and the IC₁₀₀ was 5 to 10 μg/ml except for one isolate. The MIC of miconazole was <0.03 to 16 μg/ml except for two isolates. Voriconazole had a MIC of 2 to 8 μg/ml for three isolates but >8 μg/ml for four isolates. The MIC of fluconazole was >64 μg/ml except for one isolate, and Itraconazole and micafungin had no detectable activity against the *Fusarium* spp. at concentrations of 8 (MIC), and 16 (MEC) μg/ml or lower, respectively.

There was statistically no significant difference between the antifungal agents used and clinical prognosis.

**Discussion**

This study investigated the susceptibility of *Fusarium* clinical isolates to antibacterial, biocidal, and antifungal agents. Our results showed that the antibacterial agents: gatifloxacin, levofloxacin, moxifloxacin, tobramycin,
Table 1. Demographics of patients.

<table>
<thead>
<tr>
<th>No. of case/isolate</th>
<th>Sex/ Age (yrs)</th>
<th>Side</th>
<th>Medical conditions</th>
<th>Ocular disease</th>
<th>Steroid</th>
<th>CL</th>
<th>Ocular trauma</th>
<th>Isolated organism</th>
<th>Antibacterial agent #</th>
<th>Steroid</th>
<th>Antifungal agent</th>
<th>Surgery</th>
<th>Duration from onset to beginning of antifungal treatment</th>
<th>Final BCVA</th>
<th>Occupation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M / 75</td>
<td>Right</td>
<td>DM</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Fusarium falciforme (FSSC) IFM 55596</td>
<td>IPM OFLX</td>
<td>PSL 20mg</td>
<td>AMPH-B</td>
<td>Enucleation 32</td>
<td>NLP</td>
<td>Farmer</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>M / 75</td>
<td>Left</td>
<td>DM</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Injury by corn stalks</td>
<td>Fusarium napiforme (GFSC) IFM 5802</td>
<td>MCR LVFX</td>
<td>-</td>
<td>FLCLZ ITCLZ PMC FRCZ</td>
<td>-</td>
<td>20</td>
<td>16/20</td>
<td>Farmer</td>
</tr>
<tr>
<td>3</td>
<td>F / 85</td>
<td>Right</td>
<td>-</td>
<td>Cataract/GLaucoma surgery</td>
<td>-</td>
<td>-</td>
<td>Injury by finger</td>
<td>Fusarium falciforme (FSSC) IFM 58106</td>
<td>GFLX</td>
<td>MFLX</td>
<td>OFLX</td>
<td>FLCLZ PMR VRCZ</td>
<td>-</td>
<td>29</td>
<td>14/20</td>
</tr>
<tr>
<td>4</td>
<td>M / 65</td>
<td>Right</td>
<td>DM</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Injury by finger</td>
<td>Fusarium falciforme (FSSC) IFM 59509</td>
<td>-</td>
<td>-</td>
<td>FLCLZ ITCLZ MFCG PMR</td>
<td>Enucleation 10</td>
<td>NLP</td>
<td>Office worker</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>F / 65</td>
<td>Right</td>
<td>-</td>
<td>Herpetic keratitis/Iridocyclitis</td>
<td>FLM</td>
<td>-</td>
<td>-</td>
<td>Fusarium spp. (FIESC) IFM 58306</td>
<td>LVFX</td>
<td>-</td>
<td>AMPH-B FLCLZ ITCLZ PMC FRCZ</td>
<td>Enucleation 4</td>
<td>NLP</td>
<td>Housewife</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>M / 32</td>
<td>Left</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Injury by glass</td>
<td>Fusarium falciforme (FSSC) IFM 61623</td>
<td>CMX</td>
<td>OFLX TOB</td>
<td>AMPH-B PMC FRCZ</td>
<td>-</td>
<td>6</td>
<td>30/20</td>
<td>Worker</td>
</tr>
<tr>
<td>7</td>
<td>M / 70</td>
<td>Right</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Injury by presumed soil while farming</td>
<td>Fusarium spp. (FIESC) IFM 62053</td>
<td>LVFX</td>
<td>OFLX</td>
<td>PMR VRCZ</td>
<td>-</td>
<td>11</td>
<td>16/20</td>
<td>Janitor</td>
</tr>
</tbody>
</table>

Note: Except for IPM in case 1, others were used as topical administrations; AMPH-B, amphotericin-B; BCVA, best corrected visual acuity; CL, contact lens; CMX, cefmenoxime; DM, diabetes mellitus; f-FLCZ, fos-fluconazole; FIESC, Fusarium incarnatum-equiseti species complex; FLCLZ, fluconazole; FLM, 0.1% fluorometholone; FSSC, Fusarium solani species complex; GFLX, gatifloxacin; GFSC, Gibberella fujikuroi species complex; IFM, Institute for Food Microbiology (at present, Medical Mycology Research Center, Chiba University), Chiba, Japan; IPM, imipenem; ITCLZ, itraconazole; LVFX, levofloxacin; MFCG, micafungin; MCR, micromycin; MFLX, moxifloxacin; NLP, no light perception; OFLX, ofloxacin; PMR, pimaricin; PSL, predonisolone; TOB, tobramycin; VRCZ, voriconazole.
Table 2. Susceptibility of *Fusarium* to antibacterial and biocidal agents.

<table>
<thead>
<tr>
<th>No. of isolate</th>
<th>Tested isolate</th>
<th>Flomoxef</th>
<th>Imipenem</th>
<th>Gatifloxacin</th>
<th>Levofloxacin</th>
<th>Moxifloxacin</th>
<th>Tobramycin</th>
<th>Gentamicin</th>
<th>Tobracin®</th>
<th>Benzalkonium Chloride</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IC50</td>
<td>IC100</td>
<td>IC50</td>
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<td>IC50</td>
<td>IC100</td>
<td>IC50 IC100</td>
</tr>
<tr>
<td>1</td>
<td><em>Fusarium</em> falciforme (FSSC) IFM 55596</td>
<td>&gt;5000</td>
<td>&gt;5000</td>
<td>&gt;5000</td>
<td>&gt;1500</td>
<td>625</td>
<td>&gt;2500</td>
<td>375</td>
<td>187.5</td>
<td>1500 187.5 375 4</td>
</tr>
<tr>
<td>2</td>
<td><em>Fusarium</em> napiforme (GFSC) IFM 58002</td>
<td>&gt;5000</td>
<td>&gt;5000</td>
<td>&gt;5000</td>
<td>&gt;1500</td>
<td>1250</td>
<td>&gt;2500</td>
<td>375</td>
<td>&gt;3000</td>
<td>187.5 &gt;3000 187.5 750 4</td>
</tr>
<tr>
<td>3</td>
<td><em>Fusarium</em> falciforme (FSSC) IFM 58106</td>
<td>&gt;5000</td>
<td>&gt;5000</td>
<td>&gt;5000</td>
<td>&gt;1500</td>
<td>312.5</td>
<td>&gt;2500</td>
<td>187.5</td>
<td>1500</td>
<td>93.75 1500 93.75 187.5 4 32</td>
</tr>
<tr>
<td>4</td>
<td><em>Fusarium</em> falciforme (FSSC) IFM 59509</td>
<td>&gt;5000</td>
<td>&gt;5000</td>
<td>&gt;5000</td>
<td>&gt;1500</td>
<td>312.5</td>
<td>&gt;2500</td>
<td>156.25</td>
<td>187.5</td>
<td>1500 93.75 187.5 4 128</td>
</tr>
<tr>
<td>5</td>
<td><em>Fusarium</em> spp. (FIESC) IFM 58306</td>
<td>&gt;5000</td>
<td>&gt;5000</td>
<td>&gt;5000</td>
<td>&gt;1500</td>
<td>&gt;2500</td>
<td>&gt;2500</td>
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<td>&gt;2500</td>
<td>187.5 &gt;3000 &gt;3000 187.5 750 4 256</td>
</tr>
<tr>
<td>6</td>
<td><em>Fusarium</em> falciforme (FSSC) IFM 61623</td>
<td>&gt;5000</td>
<td>&gt;5000</td>
<td>&gt;5000</td>
<td>&gt;1500</td>
<td>&gt;2500</td>
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<td>&gt;2500</td>
<td>187.5 &gt;3000 &gt;3000 187.5 750 4 256</td>
</tr>
<tr>
<td>7</td>
<td><em>Fusarium</em> spp. (FSSC) IFM 62053</td>
<td>&gt;5000</td>
<td>&gt;5000</td>
<td>&gt;5000</td>
<td>&gt;1500</td>
<td>&gt;2500</td>
<td>&gt;2500</td>
<td>&gt;2500</td>
<td>&gt;2500</td>
<td>187.5 &gt;3000 &gt;3000 187.5 750 4 256</td>
</tr>
</tbody>
</table>

Note: FIESC, *Fusarium incarnatum-equiseti* species complex; FSSC, *Fusarium solani* species complex; GFSC, *Gibberella fujikuroi* species complex; IFM, institute for food microbiology (at present, Medical Mycology Research Center, Chiba University), Chiba, Japan; IC50, the lowest concentration (μg/ml) of an agent that inhibited approximately 50% of the drug-free growth control; IC100, the lowest concentration (μg/ml) of an agent that completely inhibited the growth of *Fusarium*; Tobracin®, tobramycin 3000 μg/ml + benzalkonium chloride 25 μg/ml.
Table 3. Susceptibility of Fusarium to antifungal agents.

<table>
<thead>
<tr>
<th>No. of isolate</th>
<th>Tested isolate</th>
<th>Amphotericin B</th>
<th>Pimaricin</th>
<th>Fluconazole</th>
<th>Itraconazole</th>
<th>Miconazole</th>
<th>Voriconazole</th>
<th>Micafungin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Fusarium falciforme</em> (FSSC) IFM 55596</td>
<td>16</td>
<td>5</td>
<td>5 &gt;64</td>
<td>&gt;8</td>
<td>0.5</td>
<td>&gt;8</td>
<td>&gt;16</td>
</tr>
<tr>
<td>2</td>
<td><em>Fusarium napiforme</em> (GFSC) IFM 58002</td>
<td>1</td>
<td>5</td>
<td>5 &gt;64</td>
<td>&gt;8</td>
<td>&lt;0.03</td>
<td>8</td>
<td>&gt;16</td>
</tr>
<tr>
<td>3</td>
<td><em>Fusarium falciforme</em> (FSSC) IFM 58106</td>
<td>2</td>
<td>5</td>
<td>10 &gt;64</td>
<td>&gt;8</td>
<td>4</td>
<td>&gt;8</td>
<td>&gt;16</td>
</tr>
<tr>
<td>4</td>
<td><em>Fusarium falciforme</em> (FSSC) IFM 59509</td>
<td>1</td>
<td>5</td>
<td>10 &gt;64</td>
<td>&gt;8</td>
<td>16</td>
<td>&gt;8</td>
<td>&gt;16</td>
</tr>
<tr>
<td>5</td>
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<td>2</td>
<td>2.5</td>
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<td>32</td>
<td>&gt;8</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
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<td>&gt;16</td>
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<td>&gt;16</td>
<td>2</td>
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<tr>
<td>7</td>
<td><em>Fusarium spp.</em> (FSSC) IFM 62053</td>
<td>1</td>
<td>5</td>
<td>5 &gt;64</td>
<td>&gt;8</td>
<td>&gt;16</td>
<td>&gt;8</td>
<td>&gt;16</td>
</tr>
</tbody>
</table>

FIESC, *Fusarium incarnatum-equiseti* species complex; FSSC, *Fusarium solani* species complex; GFSC, *Gibberella fujikuroi* species complex; IC50, the lowest concentration (μg/ml) of an agent that inhibited approximately 50% of the drug-free growth control; IC100, the lowest concentration (μg/ml) of an agent that completely inhibited the growth of *Fusarium*; IFM, institute for food microbiology (at present, Medical Mycology Research Center, Chiba University), Chiba, Japan; MEC, minimum effective concentration (μg/ml); MIC, minimum inhibitory concentration (μg/ml).

Gentamicin, Tobracin®, and the biocidal agent: BAK, had inhibitory activity against the *Fusarium* spp. However, the concentrations needed were much higher than those of the antifungal agents (Tables 2 and 3). Among the antibacterial agents and the biocidal agent, BAK had the highest inhibitory activity followed by the aminoglycoside agents. Among the fluoroquinolones, moxifloxacin had the highest activity against the *Fusarium* spp.

Tobracin® with 25 μg/ml of BAK as a preservative had ≥2-fold higher activity than the IC50 and 2 to 8-fold higher activity than the IC100 of tobramycin for the *Fusarium* spp. in this study. Thimerosal and phenylmercuric acetate are also agents used in ophthalmic solutions as preservatives. These agents have been reported to have MIC of 0.0078 to 0.0625 μg/ml for *Fusarium*, *Aspergillus*, and *Alternaria* [12,13], and they might be more effective in treating fungal keratitis than BAK, natamycin, and amphotericin B. Thus, the combination of antibacterial agents and biocidal agents may have a greater inhibitory effect against the growth of *Fusarium* spp [8].

Although the IC50 and IC100 of antibacterial agents against the *Fusarium* spp. were very high in comparison to that of antifungal agents, the generally prescribed antibacterial eye drops are already at very high concentrations for bacteria. For example, Vegamox® (0.5% moxifloxacin; Alcon Co., Ltd., Tokyo, Japan), Cravit® (1.5% levofloxacin; Santen Pharmaceutical Co., Ltd., Osaka, Japan), and Riftamycin® (0.3% gentamicin and 0.02% BAK; Wakamoto Co., Ltd., Tokyo, Japan) have concentrations equal to about 4- to 32-fold, 12- to 48-fold, and 16- to 32-fold, respectively, of the IC50 for the *Fusarium* spp. In addition, the barrier function of the corneal epithelial cells with keratitis is broken, and so it would be easy for the antibacterial agents to penetrate the corneal tissue. Therefore, the combination treatments of a few antibacterial agents as initial empiric therapy and corneal scrapings might
temporally slow the progression of the disease process even in eyes with fungal keratitis.

Our results are similar to those of Day et al. [9], although there are some differences in the methods, for example, the broth used for the antibiotics and BAK for the susceptibility test, and the evaluation method for the inhibitory effects against *Fusarium* of antimicrobials. They concluded that the susceptibility of *Fusarium* to tobramycin, moxifloxacin, chloramphenicol, and BAK might explain anecdotal reports of fungal ulcers that improved with antibiotic treatment alone, and that antibiotics may have a modest effect on *Fusarium* when used as initial treatment prior to identification of the pathological organism [9].

However, the mechanisms leading to the antifungal activity of antibacterial and biocidal agents have not been elucidated. Munir et al. and Ozdek et al. suggested that the antifungal activity of the fluoroquinolones, such as moxifloxacin and levofloxacin, was through their ability to inhibit the topoisomerase enzymes [4,8]. Dalhoff reported that the aminoglycosides made fungal cell walls and membranes more permeable to ions [14]. McDonnell et al. reported that BAK was a membrane-active agent and predominantly targeted the cytoplasmic (inner) membrane of bacteria and the plasma membrane of yeasts [15]. Mehta et al. showed that amoxicillin, chloramphenicol, erythromycin, and rifacillin weakly inhibited the polygalacturonase and pectinmethygalacturonase enzyme activities of the *Fusarium* spp. in the cell wall by a degradative process [16]. Clancy et al. proposed that rifampin and rifabutin, members of the rifamycin class, and azithromycin might inhibit RNA and protein synthesis of *Fusarium* spp. when given simultaneously with amphotericin B [17,18].

Tables 2 and 3 demonstrated that among all of the antimicrobial agents, amphotericin B had the highest activity against the seven *Fusarium* spp. as has been reported [19–21]. However, we should remember that the variable susceptibility of *F. solani* species complex to amphotericin B have been reported [22,23]. Ansari et al. have reported that amphotericin B is not currently a first line agent in treating fungal keratitis given the side effect profile and lack of coverage of the different *Fusarium* species [22]. In the current study, the inhibitory effects of amphotericin B also varied for the different *Fusarium* isolates (Isolates 1 and 6, Table 3).

Pimaricin was the second most active antifungal agent for the seven *Fusarium* spp. examined in our study. Pimaricin continues to be the first line treatment in fungal keratitis and is currently considered the most effective medication against *Fusarium* [22,24]. On the other hand, miconazole and voriconazole had weak inhibitory effects which depended on the species of *Fusarium* in this study. The poor activity of voriconazole against *Fusarium* was also reported in other studies [19,20,23,25]. Prajna et al. reported that natamycin had significantly better clinical and microbiological action than voriconazole for filamentous fungal keratitis especially in cases of *Fusarium*, and stated that voriconazole should not be used as monotherapy in filamentous keratitis [25]. Sharma et al. reported that topical 1% voriconazole appeared to be a useful adjucent to natamycin in filamentous fungal keratitis including four *Fusarium* cases [26]. In this study, both pimaricin and voriconazole had been applied to five patients (Cases 2, 3, 5, 6, and 7, Table 1).

Comparing the clinical data in Table 1 and laboratory data in Table 3, we could speculate some factors related to prognosis. In the current study, three patients (Cases 1, 4, and 5, Table 1) who were treated topically or systemically with amphotericin B and/or pimaricin eventually had to undergo enucleation (Table 1). The reasons of the failure of treatment were thought as follows: a low sensitivity to amphotericin B (Isolate 1, Table 3), the non-use of amphotericin B (Case 4, Table 1), a past history of diabetes mellitus (Cases 1 and 4, Table 1), a delay in the beginning of treatment with antifungal agents (32 days in Case 1, Table 1), and the use of steroids (Cases 1 and 5, Table 1). In addition, the organisms isolated from the Cases 1 and 4 was FSSC (Tables 1 and 3). In general *F. solani* infections have been thought to be more difficult to treat and have higher MICs than non-*F. solani* cases [23,27]. In this study, some of the five FSSC had higher MIC to amphotericin B and voriconazole than non-*F. solani* isolates (Isolates 2 and 5, Table 3) but no statistically significant difference. In addition, four (Cases 2, 3, 6, and 7, Table 1) of five patients treated with both pimaricin and voriconazole had good visual acuity after treatment but any statistical significance was not found because of small sample size (P > 0.1).

Morphologic and/or molecular testing can help in distinguishing *F. solani* isolates from members of other species complexes where lower MIC values were found [19,20,28]. However, it should note that some of *Fusarium* isolates have low susceptibility or resistance (>16 µg/ml) to amphotericin B. In addition, the incidence of *Fusarium* keratitis related to contact lens wear has recently increased [3,29,30]. Therefore, in vitro susceptibility testing and identification to the species level would have great value and be essential in the treatment of *Fusarium* keratitis, although the results of susceptibility testing are not always consistent with clinical response and prognosis because antifungal agents do not have enough intracorneal and intraocular penetration [28]. If the Cases 1 and 5 (Table 1) had been treated with miconazole according to the results of susceptibility test, both might have been rescued.
Many larger studies have been done and consensus has not been reached on the best drugs to treat *Fusarium* keratitis [22]. Also, the results of this study did not find a new topically-applied treatment for *Fusarium* keratitis, although this study offered the possibility of treatment for *Fusarium* keratitis except for antifungal agents.

In conclusion, our in vitro results showed that fluoroquinolones, aminoglycosides, BAK, and Tobracin® had some inhibitory effects against the *Fusarium* spp, although antibacterial agents were considerably inferior to the antifungal agents tested. And amphotericin B had variable activity against the different *Fusarium* isolates and pimaricin is limited to topical use, although both are the most effective agents against *Fusarium* keratitis. Integrated therapy for *Fusarium* keratitis still remains to be established. Therefore, further studies for more effective medications against *Fusarium*, such as different combinations of antibacterial, biocidal, and antifungal agents are needed. Such studies should lead to the development of new agents against *Fusarium* keratitis.

**Declaration of interest**

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

**References**


