

# Microbiological profile of causes of corneal ulcers at Sultan Agung Islamic Teaching Hospital Semarang

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## ABSTRACT

### Background

Corneal ulcer is a medical condition that is the most significant cause of morbidity and blindness worldwide, caused most often by bacteria and fungi. This study aimed to determine the microbiological profile of the causes of corneal ulcers at Sultan Agung Islamic Teaching Hospital, Semarang.

### Methods

This research is a descriptive study with a cross-sectional design. Samples were all patients with corneal ulcers at Sultan Agung Islamic Teaching Hospital Semarang from July 1, 2018 – July 31, 2023; the sampling method used consecutive sampling, and the sample size was the total population. Samples were taken using a scraping on the cornea, then implanted directly in Blood Agar Plate and Sabouroud Dextrose Agar. Bacterial colonies are identified using API®, and a susceptibility test was performed on Muller Hinton Agar using the Kirby Bauer method. Fungal colonies are identified using Lacto Phenol Cotton Blue, Gram, and Germ Tube.

### Results

There were 85 samples, with no growth as many as 41 samples (48%). Fungi cause more infections than bacteria. The fungus that causes the most extensive corneal ulcers is *Aspergillus* sp as much as 13, while the group of bacteria is *Staphylococcus* coagulase negative as much as 4 (50%)

### Conclusion

Most of the microorganisms that cause corneal ulcers are fungi compared to bacteria. No Multi-Drug Resistant Organisms were found in the causative bacteria, and no azole resistance was found in fungi.

### Keywords

microbiology; corneal ulcer; Sultan Agung Islamic Teaching Hospital Semarang

## INTRODUCTION

Corneal ulcers are discontinuities or partial loss of the corneal surface due to the death of corneal tissue. The formation of corneal ulcers is due to collagenases formed by new epithelial cells and inflammatory cells. Healed corneal ulcers will cause corneal scarring and are the cause of blindness.<sup>1</sup> Epidemiological data states that corneal ulcer incidence is mainly caused by trauma followed by cornea infection. Men are more than women; the age is mostly the productive age of 21-40 years, the occupation is mostly farmer<sup>2,3</sup>. Both infectious and non-infectious agents can cause corneal ulcers. The most common corneal ulcers caused by bacteria are *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*,

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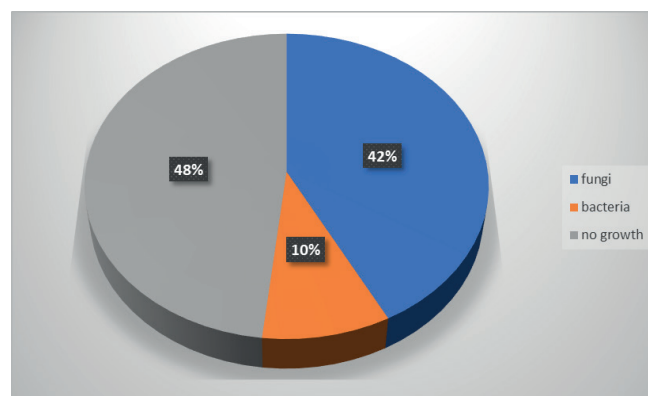
*Streptococcus pyogenes*, *Moraxella sp*, *Pseudomonas aeruginosa*, *Proteus sp*, *Klebsiella pneumoniae*, and *Escherichia coli*<sup>4,5</sup>. Fungi can also cause infection in corneal ulcers. The most extensive fungi that cause infection in the cornea are *Culvularia sp*, *Aspergillus sp*, *Paecilomyces sp*, *Cladosporium sp*, *Bipolaris sp*, *Alternaria sp*, and *Candida sp*<sup>6,7</sup>. Infection can also be caused by viruses or parasites, namely *Herpes Simplex*<sup>8</sup>, or *Acanthamoeba*<sup>9</sup>. Causes of non-infectious corneal ulcers include trauma, which is acidic or alkaline, radiation trauma, and temperature<sup>10</sup>.

Early diagnosis of lesions, profile of causative microorganisms and appropriate antimicrobial therapy can be used to control infection in lesions to prevent complications and improve the patient's quality of life. Antibiotic sensitivity testing on microorganisms that cause corneal ulcers is also necessary for infection management to carry out appropriate and accurate therapy<sup>9,11</sup>. Resistant bacteria cause some infection-causing microbes in corneal ulcers. *Pseudomonas aeruginosa* is reportedly resistant to the antibiotic moxifloxacin<sup>12</sup> and other fluoroquinolones<sup>13</sup>. The microbiological profile of one place is different from another. This study aims to determine the microbiological profile of the causes of corneal ulcers at Sultan Agung Islamic Teaching Hospital Semarang. The profile to be studied includes etiology and antimicrobial sensitivity tests.

## METHOD

This is descriptive research. The samples of this study are all patients with corneal ulcers at Sultan Agung Islamic Teaching Hospital Semarang from July 1, 2018 – July 31, 2023; the sampling method uses consecutive sampling, the sample size is the total population. Samples were taken using scrapings on the cornea, then planted directly in the medium Blood Agar Plate (bacterial growth medium) and Saboroud Dextrose Agar (fungal growth medium), and Gram and Giemsa staining are carried out. Bacterial colonies growing on the Blood Agar medium were then identified using API® and sensitivity tests were carried out on the Muller Hinton Agar medium by the Kirby Bauer method<sup>(14)</sup>. Fungal colonies that grow on Saboroud Dextrose Agar medium are followed by a mushroom identification test using LPCB (Lacto Phenol Cotton Blue) staining for mold type fungi, while yeast types are identified using Gram staining and Germ Tube.

## Result



**Graph 1.** Result on Corneal Scraping

Data from microbiological examination on corneal scraping samples between July 2018 – July 2023 were obtained as many as 85 samples, with no growth of 41 samples (48%). The causes of infection by fungi are more than bacteria. The fungus that causes the most extensive corneal ulcers is the mold group compared to the yeast group. The largest mold group is *Aspergillus sp*, while the largest yeast group is *Candida sp*. The etiological distribution of fungi, both mold and yeast groups and bacteria, is shown in Table 1.

**Table 1.** Etiology of Corneal Ulcer

Fungal	n	%	Bacteria	n	%
<i>Aspergillus sp</i> *	13	36,1	<i>Staphylococcus coagulase negative</i>	4	50
<i>Fusarium sp</i>	6	16,7	<i>Pseudomonas aeruginosa</i>	3	37,5
<i>Penicillium sp</i>	3	8,3	<i>Enterobacter cloacae</i>	1	12,5
<i>Acremonium sp</i>	3	8,3			
<i>Culvularia sp</i>	2	5,6			
<i>Alternaria sp</i>	2	5,6			
<i>Paecilomyces sp</i>	1	2,8			
<i>Trichophyton sp</i>	1	2,8			
<i>Candida sp</i>	5	13,8			
	36	100		8	100

\**Aspergillus flavus*:11, *Aspergillus fumigatus*:2

**Table 2.** Antibiotic Sensitivity Test

Bacteria	% Sensitive						
	Ampicillin	Cefotaxime	Ceftazidime	Cefepime	Ciprofloxacin	Gentamicin	Meropenem
<i>Staphylococcus coagulase negative</i>	25	100	100	100	100	100	100
<i>Pseudomonas aeruginosa</i>	0	0	100	100	100	100	100
<i>Enterobacter cloacae</i>	0	100	100	100	100	100	100
Fungi	% Sensitive						
	Fluconazole	Voriconazole					
<i>Candida sp</i>	100	100					

The results of the antibiotic sensitivity test show that many etiology-causing bacteria are still sensitive to various classes of antibiotics, for example, *Pseudomonas aeruginosa* is still sensitive to anti-pseudomonal antibiotics, such as cephalosporins (ceftazidime, cefepime), fluoroquinolone group (ciprofloxacin), aminoglycoside group (gentamicin), and carbapenem group (meropenem). *Staphylococcus coagulase-negative* bacteria are mainly resistant to ampicillin.

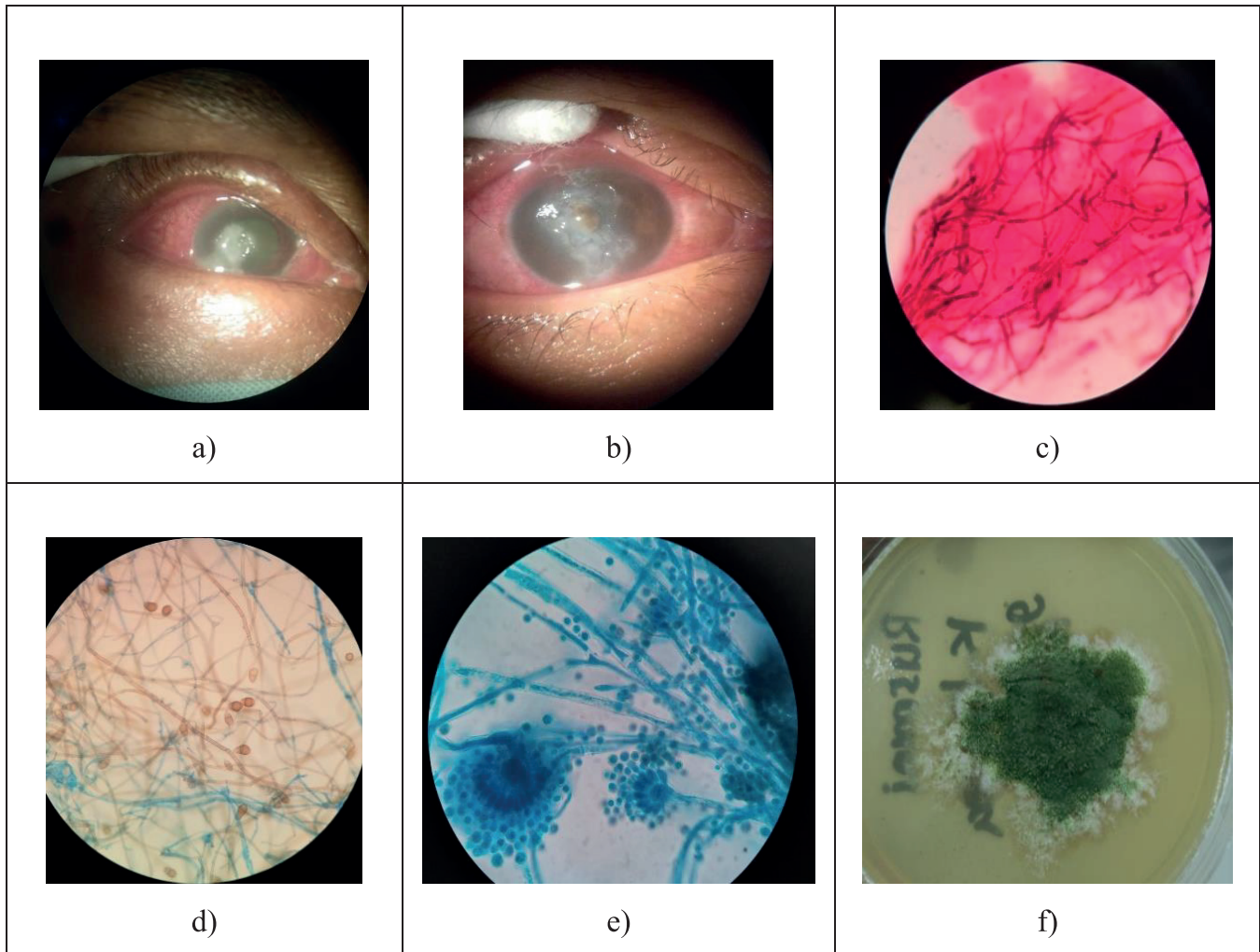
## DISCUSSION

The cause of infection in corneal ulcers can be both endogenous and exogenous. Infections of exogenous origin such as viruses, bacteria, fungi, or parasites. The pattern of microorganisms that cause keratitis varies based on variations in geography as well as local climate. Most microorganisms can invade the corneal stroma if normal corneal defence mechanisms such as lashes, tears, corneal epithelium are impaired. The pathogenicity of an organism can be demonstrated by the ability to attach to the edges or bases of epithelial defects. Bacterial toxins and enzymes a microorganism produces help digest and degrade the corneal matrix<sup>(6,15)</sup>. Complete corneal damage can occur within 24 – 48 hours, but early diagnosis based on the results of clinical examination supported by microbiological examination and prompt treatment will prevent the possibility of permanent vision loss and damage to corneal structures<sup>1</sup>. Corneal scrapings are indicated when a corneal ulcer caused by a microorganism is suspected. Early diagnostic measures also help in identifying the cause as well as selecting the right antimicrobial therapy<sup>16</sup>, It also helps speed up disease resolution by increasing antimicrobial penetration, and reducing the number of

microorganisms at the site of infection<sup>17</sup>.

The results of culture ethology of corneal ulcer causes at Sultan Agung Islamic Teaching Hospital Semarang are no different from other places, where most of them are dominated by fungi, and the most significant causes are *Aspergillus sp*<sup>3</sup>, and *Fusarium sp*<sup>4,5,7,18</sup>. Fungi are the most common cause of corneal ulcers. There are 70 species of fungi that can cause corneal ulcers, but the most common are yeast and molds. *Aspergillus* and *Fusarium* are fungi responsible for 1/3 of traumatic corneal ulcer cases, so proper diagnosis and effective therapy are needed. Predisposing factors to corneal ulcers due to fungi are mainly caused due to trauma with parts of the plant<sup>19</sup>. Trauma can damage normal defence mechanisms and allow normal conjunctival flora or pathogenic flora to enter to invade damaged corneal tissue. Tissue damage will significantly threaten vision and potential corneal perforation<sup>20</sup>. Management of fungal infections is more difficult because most antifungals are fungistatic, so they require a longer time to eradicate. The yeast group antifungal sensitivity test (*Candida sp*) results at Sultan Agung Islamic Teaching Hospital Semarang are all sensitive to azole group antifungals. This study needs to improve due to limited infrastructure for mold antifungal sensitivity tests.

The pattern of microorganisms that cause keratitis varies based on variations in geography as well as local climate<sup>21</sup>. The bacteria that cause corneal ulcers in Sultan Agung Islamic Teaching Hospital Semarang in the gram-negative group are *Pseudomonas aeruginosa*, while in the gram-positive group by *Staphylococcus coagulase negative*. The results of this study are the same as results elsewhere<sup>4,5,7,18</sup>. *Pseudomonas aeruginosa* is a causative



**Figure 1.** a) Corneal ulcers due to bacteria, b) Corneal ulcers due to fungi, c) Corneal scraping hyphae in gram staining, d) *Culvularia sp* in Lacto Phenol Cotton Blue staining, e) *Aspergillus sp* in Lacto Phenol Cotton Blue staining, f) *Aspergillus sp* colonies in saboroud dextrose agar medium

bacterium that is often found in cases of corneal ulcers. These bacteria can cause corneal perforation if the diagnosis and treatment of infection is inappropriate. The attachment of *Pseudomonas aeruginosa* to the cornea is caused by its pili containing magnesium and calcium, as well as biofilms that can damage the epithelium<sup>11</sup>. The antibiotic sensitivity pattern in the bacteria that cause corneal ulcers at Sultan Agung Islamic Teaching Hospital Semarang is still sensitive in the gram-positive and gram-negative groups. This is different from other regions, where many resistant organisms<sup>22</sup> or MDRO (Multi Drug Resistant Organism)<sup>23</sup> have begun to be found, for example *Pseudomonas aeruginosa* resistance to fluoroquinolone antibiotics<sup>24</sup>,

and the discovery of *Staphylococcus aureus* Resistant Metisillin as a cause of corneal ulcers<sup>25</sup>. 48% of samples with clinical corneal ulcers did not grow on culture. This is likely because Sultan Agung Islamic Teaching Hospital Semarang is a referral hospital, and patients have received antimicrobial therapy for a long time before being referred<sup>26</sup>.

## CONCLUSION

The microorganism that causes the most corneal ulcers is the fungus with the most isolate, namely *Aspergillus flavus*, while for the most bacteria caused by *Staphylococcus coagulase negative* in the Gram positive group, and *Pseudomonas aeruginosa* in the

gram negative group. No MDRO was found in the causative bacteria, and no azole resistance was found in the fungal sensitivity test. The limitations of this study are that it has not been able to display identification and sensitivity tests for mold class antifungals, and does not test on the causes of other microorganisms such as viruses or parasites.

### Acknowledgment

The authors would like to give the highest appreciation to the Faculty of Medicine Universitas Islam Sultan Agung for funding this research. There are no conflicts

of interests regarding this research and this research has not being published anywhere.

### Author Contribution:

Study design: Masfiah, Rahayu, Nika Bellarinasari

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Data analysis: Masfiah, Rahayu, Nika Bellarinasari

Writing and submitting the manuscript: Masfiah, Rahayu, Nika Bellarinasari, Putri R Ayuningtyas

Editing and approval of final draft: Masfiah, Putri R Ayuningtyas

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