

# Corneal ulcer: a retrospective study of a cases seen at the Hospital das Clínicas, Federal University of Espirito Santo

## *Úlcera de córnea: estudo retrospectivo de casos atendidos no Hospital das Clínicas da Universidade Federal do Espírito Santo*

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### ABSTRACT

**Objective:** To identify the major etiological agents of UC in the main referral center for this disease in the state of Espírito Santo (Hospital Universitario Cassiano Antonio de Moraes – HUCAM). **Methods:** This is a retrospective study of UC cases that underwent microbiological analysis from January 2009 to June 2013 at HUCAM. **Results:** Three hundred ninety-eight cases were studied. Microbiological cultures were positive in 60% and negative in 40% of cases. The Gram stain was positive in 28%, negative in 61% and was not performed in 11%. Among the total number of tests, 16.3% were classified as insufficient material for analysis. The microbiological examination, including gram stain and culture, was positive in 250 tests (62.8%). It was identified bacteria in 48%, fungi in 17.6% and protozoa in 0.8% of cases. **Conclusion:** The study identified the main etiological agents involved in the UC at HUCAM. Hence, it provides data that can help physicians to do a better presumptive diagnosis and a more appropriate initial empirical treatment when indicated. Gram positive bacteria and filamentous fungi have a prominent role in the etiology of UC in ES.

**Keywords:** Corneal ulcer; Eye infections

### RESUMO

**Objetivo:** Identificar os principais agentes etiológicos das úlceras de córnea atendidas no principal centro de referência para esta moléstia no estado do Espírito Santo (Hospital Universitário Cassiano Antônio de Moraes – HUCAM). **Métodos:** Estudo retrospectivo de prontuários, identificados por meio dos registros do laboratório de microbiologia do HUCAM, dos casos de úlcera de córnea submetidos à coleta de material para análise microbiológica no período de janeiro de 2009 a junho de 2013. **Resultados:** Dos 398 casos foram estudados e o resultado da cultura foi positivo em 60% e negativo em 40% dos casos. A bacterioscopia foi positiva em 28%, negativa em 61% e não foi realizada em 11%. Dentre o total de exames, 16,3% foram classificados como material insuficiente para análise. O exame microbiológico, incluindo bacterioscopia e cultura, foi positivo em 250 exames (62,8%), sendo identificado bactérias em 48% dos casos, fungos em 17,6% e protozoários em 0,8%. **Conclusão:** Este trabalho identificou os principais agentes etiológicos envolvidos na UC atendidas no HUCAM. Desta forma, fornecemos subsídios para um melhor o diagnóstico presuntivo e condução mais apropriada do tratamento empírico inicial, quando indicado. As bactérias Gram-positivas e fungos filamentosos apresentam papel de destaque na etiologia das UC no ES.

**Descritores:** Úlcera da córnea; infecções oculares

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## INTRODUCTION

Infectious corneal ulcer (ICU) or infectious keratitis is an ophthalmic emergency. It involves a loss of integrity of the corneal epithelium with infiltration of the underlying stroma by leukocytes, which are associated with inflammation. Corneal injury is caused both by the aetiological agent (fungi, bacteria, virus, or protozoa) and by immune mechanisms.

Although studies comparing the aetiology and incidence of ICUs are still limited, important variations are known to occur across continents, countries and regions<sup>1-3</sup>. The epidemiology of ICUs is not well determined because most data come from referral hospitals, therefore results may not be representative of the general population. In developing countries, 1.5 to 2 million ulcers are estimated to occur every year<sup>2</sup>, resulting in a large number of corneal opacities, which in turn represent the second leading cause of preventable blindness in certain tropical countries<sup>1</sup>.

The infectious agent is identified through microbiological analysis of a sample of the lesion<sup>1</sup>, which should always include a culture with antibiogram. However, empirical treatment with broad-spectrum topical antibiotics — based on medical history and the clinical characteristics of the lesion — must often be initiated early to control the infectious process. Prior knowledge of the epidemiological profile of infectious ulcers in the region can be very useful for choosing the type of empirical treatment. Around the world, 232 species of microorganisms that cause ICUs have been identified<sup>2</sup>. Therefore, microbiological analysis in referral hospitals is critical to identify the most prevalent aetiological agents in the region and to provide important information on their sensitivity to existing antibiotics<sup>4</sup>.

The aim of this study was to evaluate the epidemiology of infectious ulcers at Cassiano Antônio de Moraes University Hospital (HUCAM) in order to assist physicians in the clinical management of cases in the region.

## METHODS

HUCAM is a referral centre in the State of Espírito Santo (ES) for aetiological diagnosis and treatment of corneal ulcers. Patients treated at HUCAM come from all across ES and even from neighbouring states such as Bahia and Minas Gerais. Its staff has broad experience treating corneal ulcers, and the hospital also has a microbiological laboratory and an eye bank for the treatment of complicated cases that require corneal transplantation.

This was a retrospective study of all corneal ulcers submitted to microbiological analysis at HUCAM between January 2009 and June 2013. Data were obtained from the records of HUCAM's Laboratory of Microbiology. All cases described as corneal ulcers were included, totalling 398 patients. The materials was sampled according to the Department of Ophthalmology's protocol, which includes: discontinuing the use of air conditioning in the room; topical anaesthesia with instillation of hydrochloride benoxinate 0.4%; placing a blepharostat; collecting material from the margin and floor of the ulcer using a Kimura spatula under slit lamp examination; preparing two slides for bacterioscopy with Gram staining; and culture in BHI (Brain Heart Infusion), blood agar, chocolate agar, and Sabouraud agar.

## RESULTS

Of the 398 patients included in the study, 264 (66.4%) were male and 134 (33.6%) were female. Culture results were positive in 60% of cases and negative in 40%. Bacterioscopy was positive in 28% of cases, negative in 61%, and was not performed in 11%. In 16.3% of cases there was insufficient material for analysis. This percentage was 63.6% in the group that did not undergo bacterioscopy, which partially explains why the test was not performed.

Microbiological examination, including bacterioscopy and culture, yielded 250 positive results (62.8%), with bacteria being identified in 48% of the cases, fungi in 17.6% and protozoans in 0.8%. Final results are distributed as shown in Table 1.

**Table 1**  
**Results from microbiological analysis of infectious corneal ulcers.**

Infectious agent	Number of cases	%
Flamentous fungi	67	16.8
Gram negative bacteria	87	21.9
Gram positive bacteria	104	26.1
Yeasts	3	0.8
Negative	148	37.2

A greater number of fungal ulcers was identified in the months of June (nine cases) and December (10 cases). In the remaining months, the number of cases ranged from two to six, as shown in Chart 1.

**Chart 1**  
**Distribution of fungal ulcers over the year, from January 2009 to December 2012.**

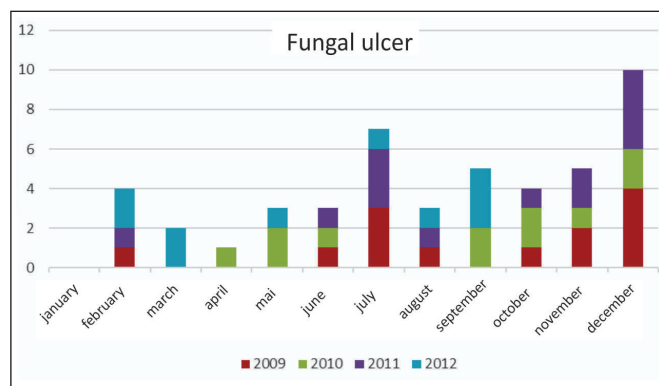


Table 2 shows the species of microorganisms found in this study.

## DISCUSSION

Around the world, infectious keratitis is a major cause of morbidity and low visual acuity due to corneal opacity, ocular perforation, and endophthalmitis<sup>1-3,5,6</sup>. The severity of the disease depends on the aggressiveness of the agent, as well as adherence

**Table 2**  
**Microorganisms found during microbiological analysis of infectious corneal ulcers**

Species	Number of cases	Species	Number of cases
<i>Achantamoeba</i>	2	<i>Proteus mirabilis</i>	1
<i>Acinetobacter baumannii</i>	2	<i>Pseudomonas aeruginosa</i>	45
<i>Aspergillus spp</i>	4	<i>Pseudomonas alcaligenes</i>	1
<i>Bacillus spp</i>	1	<i>Pseudomonas stutzeri</i>	1
<i>Bacilo Gram-negativo</i>	13	<i>Raoultella ornithinolytica</i>	1
<i>Bacilo Gram-positivo</i>	5	<i>Rizhopus spp</i>	3
<i>Burkholderia cepacia</i>	1	<i>Scedosporium spp</i>	1
<i>Candida albicans</i>	1	<i>Serratia liquefaciens</i>	1
<i>Candida spp</i>	2	<i>Serratia marcescens</i>	9
<i>Cephalosporium sp</i>	1	<i>Shewanella algae</i>	1
<i>Citrobacter koseri</i>	2	<i>Staphylococcus aureus</i>	21
<i>Cocos Gram-positivos</i>	3	<i>Staphylococcus auricularis</i>	1
<i>Curvularia spp</i>	1	<i>Staphylococcus capitis</i>	2
<i>Diplococos Gram-negativos</i>	1	<i>Staphylococcus coagulase negativa</i>	4
<i>Enterobacter cloacae</i>	1	<i>Staphylococcus epidermidis</i>	24
<i>Fungo não identificado</i>	11	<i>Staphylococcus haemolyticus</i>	1
<i>Fusarium spp</i>	43	<i>Staphylococcus warneri</i>	2
<i>Haemophilus spp</i>	1	<i>Staphylococcus hominis</i>	4
<i>Klebsiella pneumoniae</i>	1	<i>Streptococcus</i>	1
<i>Kocuria kristinae</i>	2	<i>Streptococcus agalactiae</i>	1
<i>Micrococcus spp</i>	1	<i>Streptococcus mitis</i>	3
<i>Moraxella group-</i>	2	<i>Streptococcus pneumoniae</i>	18
<i>Morganella morganii</i>	2	<i>Streptococcus viridans</i>	8
<i>Mucor spp</i>	1	<i>Trichosporon spp</i>	1
<i>Neisseria spp</i>	2	<i>Trichosporum asabii</i>	1
<i>Nocardia</i>	1	<b>Total</b>	<b>263</b>

to treatment and how soon the therapy is started. Delays in treatment are generally associated with limited access to specialist care, which is common in developing countries<sup>2,7</sup>.

The failure to provide early specific treatment against ICU can trigger severe ocular complications such as descemetocoele, perforation, and endophthalmitis. Correct identification of the aetiological agent is extremely important for selecting the specific treatment for each case. Therefore, assessing the epidemiology and seasonal variation of ICUs can help ophthalmologists determine the most likely agent and select the initial therapeutic method when microbiological analysis is not available or while waiting for culture results.

In this study, an average of 7.5 corneal ulcers were treated per month, with a predominance of male patients (66.3%, vs. 33.7% female), similar to other studies in literature<sup>8,9</sup>. For example, Sacramento et al. (2005) studied 132 patients with ICUs, of which 59.1% were male and 40.9% were female. Similarly, Saha et al. (2009) studied 74 patients, of which 65% were male and 35% were female<sup>8,9</sup>. This is probably due to fact that men are more exposed to eye injuries than women, especially rural workers<sup>9</sup>. A study in Philadelphia found a greater proportion of women with eye injuries (58.3%), although the sample size was smaller (24 cases)<sup>10</sup>. In Nepal, the proportion of male and female individuals with ICUs was similar<sup>1</sup>.

In the present study, microbiological examination was positive in 62.8% of cases, which is not ideal, although higher than in previous Brazilian (28.86 to 65%)<sup>8,12</sup> and international (40 to 80%) studies<sup>11-16</sup>. The distribution of aetiological agents

was in contrast with the literature. Among positive results, bacteria were identified in 191 (76.4%) cases (104 Gram-positive and 87 Gram-negative bacteria), fungi in 70 (28%) (of which only three were yeasts), and bacteria and protozoa in two cases (0.8%). On the other hand, in a study conducted in Sorocaba, Brazil, Rocha et al. (2011) found bacteria in 95.33% of cases (73.23% Gram-positive and 26.77% Gram-negative) and fungi in only 4.67%<sup>12</sup>. In São Paulo, Sacramento et al. (2005) found bacteria in 70.9% (51.1% Gram-positive cocci, 34.9% Gram-negative cocci, and 6.9% Gram-positive bacilli), protozoa in 16.3% and fungi in 12.8%<sup>8</sup>. In India, only 23.4 to 32.77% of ICUs are caused exclusively by bacteria, 26.43 to 34.4% by fungi, 1.04 to 1.4% by protozoa with or without bacteria, and 23.4% by microsporidia with or without bacteria<sup>14,16</sup>. In Nepal, bacteria are responsible for 63.2% of cases and fungi for 6.7%<sup>11</sup>.

There are differences in the national and international literature regarding the distribution of species, not only in comparison with our results, but also between other studies. These differences are probably due to the climate, environment, predominant economic activity and exposure to risk factors, especially trauma and the use of contact lenses<sup>1-3,12</sup>. These factors explain why developing countries, many of which have tropical climates and agriculture-based economies, present a higher percentage of fungal ulcers, in contrast with developed countries, where ulcers related to contact lenses are more common<sup>1-3</sup>. In fact, climate and geographic location are believed to be associated with the clinical presentation and progression of ulcers, especially in the case of fungi<sup>2</sup>. The most commonly isolated species in our

study were: *Pseudomonas aeruginosa* (18%), *Fusarium* spp. (17.2%), *Staphylococcus epidermidis* (9.6%), *Staphylococcus aureus* (8.4%), *Streptococcus pneumoniae* (7.2%), and *Serratia marcescens* (3.6%). The main species isolated in a study in Sorocaba were *S. aureus*, *S. epidermidis* (30.56% each), *Streptococcus* sp. and *Pseudomonas* sp. (9.43% each)<sup>12</sup>. In the city of São Paulo, the most frequent bacteria were coagulase-negative staphylococci (30.2%), *Acanthamoeba* sp. (16.3%), *Pseudomonas* sp. and *S. pneumoniae* (11.6% each)<sup>8</sup>. These results underline the significant variation between national studies.

There are also great differences in international literature concerning the aetiology of ICUs, with *S. epidermidis*, *S. pneumoniae*, and *P. aeruginosa* being the bacteria most commonly isolated<sup>2</sup>. In Nepal, bacteria accounted for 63.2% and fungi for 6.7% of cases. Also in Nepal, Upadhyay et al. found *S. pneumoniae* in 31.1% of positive cultures, followed by *S. epidermidis*, *S. aureus*, and *Pseudomonas* sp.<sup>11</sup>. In the United States, Wahl et al. found *S. epidermidis* and *S. aureus* as the major aetiological agents (28% and 16%, respectively)<sup>17</sup>.

In our study, fungal ulcers accounted for 17.6% (70) of cases, with *Fusarium* spp. and *Aspergillus* spp. being the most commonly isolated species (17.2% and 1.6% of positive cases, respectively). In Sorocaba, *Fusarium solani* was the most common fungus<sup>8</sup>. Despite the high incidence of fungal ulcers in the state of Espírito Santo, it is still below the values found in India (38.06 to 44%), Bangladesh (36%), Ghana (37.6%) and southern Florida (35%)<sup>19</sup>. Our study is in agreement with the international literature as regards the species of fungi found, with filamentous fungi, especially *Fusarium* spp. and *Aspergillus* spp., being the most common<sup>1,2,14,18</sup>. In India and Nepal, however, different results were found, with *Aspergillus* spp. being the most common followed by *C. albicans*, while *Fusarium* sp. was only the third most common fungal agent<sup>9,11</sup>. Also in India, Gopinathan et al. (2002) found *Curvularia* as the most common fungal agent<sup>19</sup>. In certain countries, yeasts account for the largest number fungal ICUs. For example, in Victoria, Australia, *C. albicans* was the most common agent (37.2%), followed by *Aspergillus fumigatus* (17.1%) and *Fusarium* sp. (14.3%)<sup>20</sup>. In Pennsylvania, United States, *C. albicans* (45.8%) was also found more frequently than *Fusarium* sp. (25%)<sup>10</sup>.

In our study, fungal ulcers occurred predominantly in the months of June and December. The higher incidence in June may be due to the fact that the state is a large coffee producer, whose harvest begins in early June. During this time of year, rural workers, who are mostly male, are exposed to eye injuries caused by certain parts of the plant<sup>9</sup>. The higher incidence in December is possibly a result of patient referrals from other clinics and municipalities that discontinue or reduce patient admissions during the holiday season. In India, a higher incidence of fungal ulcers was also documented in the monsoon season and in winter compared to the summer<sup>19</sup>.

## CONCLUSION

This study identified the main aetiological agents of ICUs treated at HUCAM, the referral hospital in the state Espírito Santo for this condition. These results can help physicians perform a better presumptive diagnosis and a more appropriate empirical treatment when indicated. Gram-positive bacteria and

filamentous fungi play a significant role in the aetiology of ICUs in the state of Espírito Santo.

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