RBPS Revista Brasileira de Pesquisa em Saúde, 2023;25(supl.2)
https://doi.org/10.47456/rbps.v25isupl_2.40856

ORIGINAL ARTICLE

Etiology, antimicrobial sensitivity test and epidemiological profile of infectious keratitis in a university reference service in the Espírito Santo State, Brazil

Etiologia, teste de sensibilidade antimicrobiana e perfil epidemiológico das ceratites infecciosas em serviço universitário de referência no estado do Espírito Santo, Brasil

Maithê Silva de Moraes[1], Ester Gonçalves de Carvalho[1], Patricia Grativol Costa Saraiva[1], Amanda Silva Guimarães[1], Rodrigo Villas Bôas Drumond[1], Kahlil Ruas Ribeiro Mendes[1], Thiago George Cabral Silva[1], Fábio Petersen Saraiva[1]

[1] Universidade Federal do Espírito Santo. Vitória/ES, Brasil.

Correspondence:
maithesmoraes@gmail.com

Subject:
24/3/2023

Approved:
8/6/2023

ISSN:
2446-5410

ABSTRACT

Introduction: Infectious keratitis (IK) or infectious corneal ulcer is an ophthalmic emergency characterized as a loss of integrity of the corneal epithelium with infiltration of the underlying stroma by leukocytes, which are associated with inflammation. **Objective**: To identify the epidemiological profile, major etiological agents and antimicrobial sensitivity test of infectious keratitis in the main reference center for this disease in the Espírito Santo State (Hospital Universitario Cassiano Antonio de Moraes - HUCAM). **Methods**: Retrospective study of infectious keratitis cases with microbiological analysis from March 2016 to March 2020 at HUCAM. **Results**: Total of 473 cases of keratitis was diagnosed during the study period and 43 patients without culture. Microbiological cultures were positive in 63.5% exams (273). Only 0.2% of the exams were classified as contamination. The main results were Gram negative bacteria in 27.7% of the cases, Gram positive bacteria in 21.4%, fungi in 16.9% and protozoa in 0.2%. **Conclusion**: The study identified the profile and analyzed the antimicrobial susceptibility of the main etiological agents with infectious keratitis attended at HUCAM. Hence, it provides data that can help ophthalmologists to obtain a better presumptive diagnosis and a more appropriate initial empirical treatment when indicated.

Keywords: Keratitis; Cornea; Corneal ulcer; Microbiology; Eye infections

RESUMO

Introdução: A ceratite infecciosa (CI) ou úlcera infecciosa da córnea é uma emergência oftálmica caracterizada como perda da integridade do epitélio da córnea com infiltração do estroma subjacente por leucócitos, que estão associados à inflamação. **Objetivo**: Identificar o perfil epidemiológico, principais agentes etiológicos e teste de sensibilidade antimicrobiana das ceratites infecciosas atendidas no principal centro de referência para esta doença no Estado do Espírito Santo (Hospital Universitário Cassiano Antônio Moraes - HUCAM). **Métodos**: Estudo retrospectivo de prontuários, identificados por meio dos registros do laboratório de microbiologia do HUCAM, dos casos de ceratite bacteriana submetidos à coleta de material para análise microbiológica no período de março de 2016 a março de 2020. **Resultados**: Foram estudados 473 casos de ceratite bacteriana durante este período e em 43 pacientes não foi realizada cultura. 273 (63,5%) culturas apresentaram resultado positivo. Apenas 0.2% dos exames foram classificados como contaminação da cultura. Foram identificados, principalmente, bactérias Gram negativas em 27.7% dos casos, bactérias Gram positivas em 21.4%, fungos em 16.9% e protozoários em 0.2%. **Conclusão**: Este trabalho identificou os principais agentes etiológicos envolvidos nas ceratites infecciosas atendidas no HUCAM, além de registrar o perfil epidemiológico e a análise microbiológica destes agentes. Desta forma, fornecemos subsídios para um melhor diagnóstico presuntivo e condução mais apropriada do tratamento empírico inicial, quando indicado.

Palavras-chave: Ceratite; Córnea; Úlcera de córnea; Microbiologia; Infecções oculares.

INTRODUCTION

Infectious keratitis (IK) or infectious corneal ulcer is an ophthalmic emergency characterized as 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 microorganisms (fungi, bacteria, virus, or protozoa) and by immune mechanisms. Around the world, 232 species of microorganisms that cause IKs have been identified.

Active corneal infection triggers inflammatory and immune responses to preserve ocular integrity, which may lead to loss of transparency and regularity of cornea. In view of this, visual acuity decreases in a large percentage of cases due to corneal scars.

The treatment must often be initiated early to control the infectious process, reducing possible visual sequelae. Therefore, the prior knowledge of the epidemiological profile of infectious keratitis in the region, such as sensitivity and resistance pattern of these etiological agents to existing antibiotics, can be very useful for choosing the initial treatment with antibiotics, sincethe therapy must be initiated before the result of culture and antibiogram, based in the anamneses and the aspects of the corneal injuries. Therefore, providing such information about etiological agents and antimicrobial sensitivity is extremely relevant for the ophthalmologist, as it provides technical support for daily medical practice.

The aim of this study was to evaluate the aetiology of infectious ulcers and stablish the resistance pattern of the microbial agents in the most important reference service of ICU in the Espírito Santo State at Hospital Universitário Cassiano Antônio Moraes (HUCAM).

METHODS

Retrospective study involving patients diagnosed with infectious keratitis in the ophthalmology urgency sector of HUCAM from March 2016 to March 2020. Data were obtained from the records of HUCAM's Laboratory of Microbiology in the time under consideration, totaling 473 patients. In 43 of these patients culture was not processed.

The collection of the materials was executed according to the service protocol, which includes: discontinuing the use of air conditioning in the room; topical anesthesia 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.

Once collected, the samples were immediately sent to the HUCAM's Laboratory of Microbiology for analysis and antimicrobial sensitivity testing (TSA).

RESULTS

A total of 473 medical records were evaluated. Three hundred and eight (65.1%) patients were male and 165 (34.9%) female. The average age of the patients evaluated was 47.83 years (standard deviation of 19.34 years).

The origin of the patients was from the metropolitan region of Vitória (State capital) 69.5% (210) and the North 7.0% (21), Northwest 7.0% (21), South 7.0% (21) regions of Espírito State. And 8.6% of patiens (26) come from other states 8.6% (26). One hundred and seventy-one patients did not present this information.

Regarding the profession, 62.3% (152) were active workers, 23.0% (56) retired, 10.2% (25) students, 4.5% (11) unemployed. Two hundred and twenty-nine patients did not present this information.

We observed that the trauma groups show significant difference in relation to gender, origin, profession and professional activity.

We too observed that in the trauma group we have a significantly greater difference in male patients than in the trauma-free group, where we have a higher number of female patients. Regarding origin, we observed a greater association of trauma cases in the South, North and Northeast regions.

Regarding the predominance of etiological agents, in female and male genders, Pseudomonas (56.7%) and Fusarium (31.5%) were observed, respectively. Data are shown in the Table 1.

TABLE 1. Absolute and relative frequencies of the most frequent agents, according to gender

	FEMALE		MALE		
ETIOLOGICAL AGENTS	**N**	**%**	**N**	**%**	**P* <0.001**
Pseudomonas	38	56.7	35	27.6	
Staphylococcus	14	20.9	36	28.4	
Fusarium	6	9.0	40	31.5	
Serratia	4	6.0	9	7.1	
Streptococcus	5	7.5	7	5.5	

* Descriptive level of probability of the chi-square test. Source: Developed by the authors.

A history of trauma was reported by 92 patients (30.3%), half of the cases (46 patients) the trauma was work-related. In these cases, no significant difference was observed in relation to age. The trauma associated with work was more present in males (59%), rural workers, construction and transportation.

The groups that reported a history of trauma showed a significant difference in relation to the findings of positive culture, presenting a significantly higher prevalence of filamentous fungi, especially the genus Fusarium, distinguishing itself from patients without a history of trauma in which a significantly higher frequency of Gram-negative bacteria was observed, with the predominant genus Pseudomonas (Table 2).

TABLE 2. Absolute and relative frequencies of the most frequent etiological agents, according to the trauma group

	TRAUMA				
	NO		YES		
ETIOLOGICAL AGENTS	**N**	**%**	**N**	**%**	**P* 0.008**
Pseudomonas	46	48.9	4	15.4	
Staphylococcus	22	23.4	7	26.9	
Fusarium	16	17.0	9	34.6	
Serratia	7	7.5	2	7.7	
Streptococcus	3	3.2	4	15.4	

* Descriptive level of probability of the chi-square test.Source: Developed by the authors.

The use of contact lens was reported by 107 patients in the study (34.9%), being proportionally higher in women (55/165) than in men (52/308). In the ulcers that occurred in contact lens users, a higher frequency of Gram-negative bacteria was recorded, especially the genus Pseudomonas (Table 3).

TABLE 3. Absolute and relative frequencies of the most frequent agents, according to the contact lens use group

	CONTACT LENS				
	NO		YES		
ETIOLOGICAL AGENTS	**N**	**%**	**N**	**%**	**P* <0.001**
Pseudomonas	14	20.0	36	72.0	
Staphylococcus	21	30.0	9	18.0	
Fusarium	22	31.4	3	6.0	
Serratia	7	10.0	2	4.0	
Streptococcus	6	8.6	0	0.0	

* Descriptive probability level of the chi-square test maximum likelihood ratio. Source: Developed by the authors.

Culture collection was not performed in forty-three (9.1%) patients.

In 152 (32.1%) patients, antimicrobial sensitivity testing was performed and the main results are shown in the table Table 4.

DISCUSSION

This study showed data that are compatible with the literature. Among the cultures performed in this study, 273 patients (63.5%) were positive, a similar result found in the literature. Sacramento *et al.*[1] for example, evaluated 133 cultures, with 65% of the cultures being positive. Comarella *et al.*[2] evaluated 398 cultures and 62.8% were positive. Green *et al.*[3] evaluating 253 cultures, 65% positive. Marujo *et al.*[4] evaluating 1.468 cultures, 71,6% positive. With the average age compatible as well.

According to other authors, we observed that infectious keratitis develops mainly from bacterial agents, which is more common when associated with the use of contact lenses in females. In these cases, the most frequently isolated agent was Gram negatives,

TABLE 4. Absolute and relative frequencies of antibiotic sensitivity/resistance according to the most frequent etiological agents

		PSEUDOMONAS		STAPHYLOCOCCUS		SERRATIA		STREPTOCOCCUS	
ANTIBIOTIC		**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Penicillin	Sensitive	19	100.0	2	40.0	3	100.0	7	100.0
	Resistant	0	0.0	3	60.0	0	0.0	0	0.0
Ampicillin	Sensitive	19	51.4	2	100.0	3	100.0	0	-
	Resistant	18	48.6	0	0.0	0	0.0	0	-
Amikacin	Sensitive	49	98.0	2	100.0	6	100.0	0	-
	Resistant	1	2.0	0	0.0	0	0.0	0	-
Aztreonam	Sensitive	24	100.0	2	100.0	3	100.0	0	0.0
Ampicillin/Sulbactam	Sensitive	19	54.3	2	100.0	3	100.0	0	-
	Resistant	16	47.7	0	0.0	0	0.0	0	-
Benzylpenicillin	Sensitive	20	100.0	2	18.2	3	100.0	0	-
	Resistant	0	0.0	9	81.8	0	0.0	0	-
Cefoxitin	Sensitive	19	51.4	2	100.0	3	50.0	0	-
	Resistant	18	48.6	0	0.0	3	50.0	0	-
Cefepime	Sensitive	45	100.0	2	100.0	6	100.0	0	100.0
Ceftazidime	Sensitive	45	100.0	2	100.0	6	100.0	0	-
	Resistant	0	0.0	0	0.0	0	0.0	0	-
Ceftriaxone	Sensitive	18	48.6	2	100.0	6	100.0	0	-
	Resistant	19	51.4	0	0.0	0	0.0	0	-
Cefuroxime	Sensitive	20	54.1	2	100.0	3	50.0	0	-
	Resistant	17	45.9	0	0.0	3	50.0	0	-
Ciprofloxacin	Sensitive	47	100.0	11	78.6	6	100.0	0	0.0
	Resistant	0	0.0	3	21.7	0	0.0	1	100.0
Clindamycin	Sensitive	19	100.0	13	100.0	3	100.0	0	-
	Resistant	0	0.0	0	0.0	0	0.0	0	-
Colistin	Sensitive	38	100.0	2	100.0	3	60.0	0	-
	Resistant	0	0.0	0	0.0	2	40.0	0	-
Erythromycin	Sensitive	19	100.0	9	69.2	4	100.0	0	-
	Resistant	0	0.0	4	30.8	0	0.0	0	-
Ertapenem	Sensitive	19	100.0	2	100.0	4	100.0	0	0,0
ESLB	Sensitive	19	100.0	2	100.0	2	50.0	0	-
	Resistant	0	0.0	0	0.0	2	50.0	0	-
Gentamicin	Sensitive	47	100.0	12	92.3	6	100.0	1	100.0
	Resistant	0	0.0	1	7.7	0	0.0	0	0.0
Imipenem	Sensitive	47	100.0	2	100.0	3	100.0	0	0.0
Meropenem	Sensitive	45	100.0	2	100.0	6	100.0	0	0.0
Linezolid	Sensitive	19	100.0	13	100.0	3	100.0	0	0.0
Levofloxacin	Sensitive	27	100.0	2	100.0	3	100.0	0	0.0

* to be continued.

* continuation.

Moxifloxacin	Sensitive	19	100.0	11	84.6	3	100.0	0	-
	Resistant	0	0.0	2	15.4	0	0.0	0	-
Norfloxacin	Sensitive	19	100.0	3	100.0	3	100.0	0	0.0
Oxacillin	Sensitive	19	100.0	32	78.0	3	100.0	0	-
	Resistant	0	0.0	9	22.0	0	0.0	0	-
Piperacillin/Tazobactam	Sensitive	48	100.0	2	100.0	3	100.0	0	0.0
Polymyxin	Sensitive	23	100.0	2	100.0	3	100.0	0	0.0
Rifampicin	Sensitive	19	100.0	13	100.0	3	100.0	0	0.0
Streptomycin	Sensitive	19	100.0	2	100.0	3	100.0	0	0.0
Teicoplanin	Sensitive	19	100.0	13	100.0	3	100.0	0	0.0
Tetracycline	Sensitive	19	100.0	2	100.0	3	100.0	0	0.0
Tigecycline	Sensitive	19	100.0	13	100.0	5	100.0	0	0.0
Trimethoprim/Sulfamethoxazole	Sensitive	19	100.0	14	100.0	3	100.0	0	0.0
Vancomycin	Sensitive	19	100.0	14	100.0	3	100.0	1	100.0

Source: Developed by the authors.

mainly Pseudomonas sp. Green *et al.*[3] demonstrated that Pseudomonas sp. was isolated in about 55% of cultures of infectious keratitis contact lens users. In our study we demonstrated that this agent was the most prevalent, was the most related of females. This reasoning is essential for defining the therapy.

The main etiological agent isolated in fungal keratitis was the filamentous fungus of the genus Fusarium sp. This information is compatible with previous national studies and demonstrate the importance to know of the epidemiological profile of the patients (middle age, trauma, work active males and rural environment) to choose the most accurate therapy. These characteristics are similar to the study of Salera *et al.*[5], Evaluating 20 patients with fungal keratitis presents that 75% were male, 60% were rural workers, at the average age of 35,7, 60% were trauma related and the main etiologic agent was Fusarium sp. (60%), followed by Aspergillus sp. (30%) and Acremonium sp. (5%). A study with conflicting results was that of Saha *et al.*[6] highlighted that the main etiologic agent of fungal keratitis was the Aspergillus genus (54.4%) and not the Fusarium genus.

Comarella *et al.*[2] identified it as the most isolated species among the cultures evaluated fungi of the genus Fusarium and Aspergillus, with 17.2% and 1.6%, being a result more compatible with this study. Marujo *et al.*[4] reported that 60% of the evaluated fungi cultures, the predominant etiological agent was Fusarium (68/116).

In addition to the importance of tracing the epidemiological profile of these agents in order to indicate the most appropriate therapy, we provide an additional data that is hardly found in the literature, the microbiological analysis with microbial sensitivity testing of these agents causing infectious keratitis.

Marta *et al.*[7] described only the resistance presented to antibiotics in the empirical treatment, without specifying the resistance pattern of the etiologic agent in question after the analysis of the antibiogram. Previous learning of the most common etiologic agents according to the patient's profile, along with knowledge of the resistance profile expected for etiological agents, are very useful tools for indicating the most appropriate treatment for each case.

Being this study characterized as retrospective, there are limitations regarding the standardization of data collection and the pattern of analysis of the antibiogram. We identified that the kits acquired by the laboratory to perform the TSA suffered variations over the period studied. In this study, the clinical aspects of infectious corneal ulcers and pre- and post-treatment visual acuity data were not

addressed, in addition to the follow-up of patients who evolved with a surgical outcome.

CONCLUSION

This study identified the main etiological agents of ICUs treated at HUCAM from March 2016 to March 2020. We analyzed the demographic characteristics of patients and the etiological profile of this condition, highlighting the prevalence of bacteria, especially Pseudomonas and Staphylococcus genus, and filamentous fungi, Fusarium genus, in addition to the study of the resistance pattern of these agents. These results can help ophthalmologists to perform a better presumptive diagnosis and a more appropriate empirical treatment when indicated.

REFERENCES

1. Sacramento RS, Castro L, Freitas D, Branco BC, Lima ALH, Vieira L, et al. Estudo dos fatores epidemiológicos e influentes na Ceratite microbiana em serviço universitário. Rev Bras Oftalmol. 2005;64(1):7-13
2. Comarella Júlia Dutra, Saraiva Patricia Grativol Costa, Saraiva Fábio Petersen. Úlcera de córnea: estudo retrospectivo de casos atendidos no Hospital das Clínicas da Universidade Federal do Espírito Santo. Rev. bras.oftalmol. [Internet]. 2015 Apr [cited 2021 May 01] ; 74(2): 76-80.
3. Green, M., Apel, A., & Stapleton, F. (2008). Risk Factors and Causative Organisms in Microbial Keratitis. Cornea, 27(1), 22–27. doi:10.1097/ico.0b013e318156caf2
4. Marujo, Fábio Iglesias; Hirai, Flávio Eduardo; Yu, Maria Cecília Zorat; Hofling-Lima, Ana Luísa; Freitas, Denise & Sato, Elcio Hideo. Distribuição das ceratites infecciosas em hospital terciário no Brasil. Arq Bras Oftalmol. 2013;76(6):370-3
5. Salera, Cristina Moreira; Tanure, Marco Antônio Guarino; Lima, Wellington Tadeu Montenegro; Campos, Christian Marcellus; Trindade, Fernando Cançado & Moreira, Júlia dos Anjos. Perfil das ceratites fúngicas no Hospital São Geral Belo Horizonte – MG. Arq Bras Oftalmol. 2002;65:9-13
6. Saha S, Banerjee D, Khetan A, Sengupta J. Epidemiological profile of fungal keratitis in urban population of West Bengal, India. Oman J Ophthalmol. 2009;2(3):114-8
7. Marta, Ana, Silva, Nisa, Carneiro, Inês, Neves, Miguel Mesquita, Gomes, Miguel, & Oliveira, Luís. (2019). Estudo epidemiológico das queratites infeciosas internadas num centro hospitalar terciário - revisão de 5 anos. Revista Brasileira de Oftalmologia, 78(6), 370-374.

STATEMENTS

Author contributions

All authors contributed equally to the writing, methodology, data collection, research, review, and final editing of this article.

Funding

The article was self-funded.

Conflict of interest

The authors declare no conflicts of interest.

Availability of research data and other materials

Research data and other materials can be obtained by contacting the authors.

Managing Editors

Carolina Fiorin Anhoque, Blima Fux, José Geraldo Mill, Tania Reuter.

Address for correspondence

Av. Mal. Campos, 1355, Santos Dumont, Vitória/ES, Brasil, CEP: 29041-295.