Microbiological and epidemiological study of infectious keratitis in children and adolescents

Estudo microbiológico e epidemiológico da ceratite infecciosa em crianças e adolescentes

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ABSTRACT

Purpose: To analyze epidemiological and microbiological aspects of microbial keratitis in children and adolescents.

Methods: This retrospective cohort study was conducted at the Department of Ophthalmology and Visual Science, Escola Paulista de Medicina, Universidade Federal de São Paulo, between July 15, 1975, and December 31, 2010. We analyzed corneal samples from 859 patients with clinical suspicion of infectious keratitis, comparing epidemiological and microbiological characteristics of bacterial keratitis with those of non-bacterial and non-viral keratitis. We also compared Gram-positive and Gram-negative pathogens in patients with bacterial keratitis. We created a susceptibility profile of the bacterial microorganisms studied.

Results: Of the 859 patients, 346 (40.3%) showed positive culture results for non-viral microorganisms. Teenagers (13-18 years) made up the group with the highest number of patients with keratitis (164, 47.4%). The most frequent risk factors for keratitis were trauma (33.5%) and previous ocular surgery (24.9%). Gram-positive bacteria (71.8%) were the most often isolated, with coagulase-negative *Staphylococcus* (23.8%) the most prevalent microorganism. Logistic regression analysis showed age (p=0.002), topical antimicrobial drug use (p=0.01), and trauma due to non-chemical burns (p=0.005) were risk factors for non-bacterial keratitis. Age (p=0.01) was also a risk factor for Gram-negative bacterial keratitis.

Conclusion: Our study showed that in the age range studied, the prevalence of keratitis caused by Gram-negative bacteria or by the non-viral microorganisms evaluated increases with age. Previous use of topical antimicrobial drug and trauma due to non-chemical burns are associated with non-bacterial keratitis. Knowledge of the risk factors and the microorganisms involved may help improve treatment of keratitis in children and adolescents and minimize visual impairment.

Keywords: Keratitis/microbiology; Keratitis/epidemiology; Eye infections; Children; Adolescent

RESUMO

Objetivos: *Descrever o perfil epidemiológico e microbiológico de ceratite microbiana em crianças e adolescentes.*

Métodos: *Estudo retrospectivo tipo coorte, utilizando fichas laboratoriais de pacientes, atendidos no Departamento de Oftalmologia e Ciências Visuais - Escola Paulista de Medicina - Universidade Federal de São Paulo, entre 15 de julho de 1975 a 31 de dezembro de 2010. Foram comparados pacientes com ceratite bacteriana e não bacteriana (não viral). Entre os pacientes com ceratite bacteriana, foram comparados aqueles em que a ceratite foi causada por bactérias Gram positivas e Gram negativas. O perfil de sensibilidade dos microrganismos bacterianos aos antimicrobianos também foi estudado.*

Resultados: *Foram analisadas amostras corneanas de 859 pacientes com suspeita clínica de ceratite infecciosa, na faixa etária estudada. Destes, 346 (40,3%) apresentaram resultados de culturas positivas para microrganismos não virais. Adolescentes (13 a 18 anos) compuseram o grupo com maior número de pacientes com ceratite (164-47,4%). Os principais fatores de risco foram trauma (33,5%) e cirurgias oculares prévias (24,9%). Bactérias Gram positivas foram isoladas com maior frequência (71,8%), sendo prevalente o patógeno* Staphylococcus *coagulase negativo (23,8%). De acordo com a análise de regressão logística, idade (p=0,002), uso tópico de drogas antimicrobianas (p=0,01) e trauma por queimadura não química (p=0,005) foram fatores predisponentes para ceratite não bacteriana. Idade (p=0,01) também foi fator de risco para ceratite causada por bactérias Gram negativas.*

Conclusões: *Nosso estudo mostrou que quanto maior a idade, na faixa etária estudada, maior a probabilidade da ceratite ser causada por bactérias Gram negativas e/ou por outros microrganismos não virais avaliados. O uso tópico de drogas antimicrobianas prévias e trauma devido à queimadura não química predispõe à ceratite não bacteriana. O conhecimento dos fatores de risco e dos microrganismos envolvidos resultarão em tratamento específico da ceratite em crianças e adolescentes, com menores danos visuais.*

Descritores: *Ceratite/microbiologia; Ceratite/epidemiologia; Infecções oculares; Criança; Adolescente*

INTRODUCTION

Infectious keratitis is rare but potentially serious disease found mainly in young people. It is a preventable cause of visual impairment, similar to including amblyopia in children and mono-or bilateral blindness [1-3].

More than one-third of eye injuries among children and adolescents result from their natural curiosity, immature motor skills, and tendency to imitate adult behavior without assessing the risks relevant to their actions. Although infectious keratitis is not common in this age group, it is potentially devastating as approximately, considering thet 30% of young victims of serious eye injuries end up with visual acuity lower than 20/200[4-7]. Infectious keratitis are more prevalent in tropical developing countries with poor healthcare systems than in developed countries[3,8-21].

The diagnosis of keratitis is usually clinical and the treatment is empirical, with the application of broad-spectrum topical antimicrobial agents until identification of the etiologic agent is completed. However, few studies have investigated the causative agents and risk factors of infectious keratitis in children and adolescents.

The purpose of this study was to investigate the epidemiological and microbiological findings of infectious keratitis in patients up to 18 years of age attending a tertiary referral center. In addition, the

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risk factors associated with the presence of bacterial keratitis and non-bacterial non-viral keratitis were analyzed, as well as the *in vitro* susceptibility profile of the bacterial isolates.

METHODS

This retrospective cohort study assessed microbial keratitis in patients up to 18 years old in the period from July 15, 1975, to December 31, 2010. All data included in the laboratory records of patients with clinical suspicion of keratitis were evaluated at the Ocular Microbiology Laboratory, Department of Ophthalmology, Federal University of São Paulo, São Paulo, Brazil. The variables studied included age, sex, affected eye, use of contact lenses, use of topical medications, ocular antecedents, comorbidities, and previous ocular surgery. The *in vitro* susceptibility profiles of the bacteria were identified, and the annual incidence of infectious keratitis and the prevalence of etiologic agents were also evaluated. The local Ethics Committee approved this study.

All subjects underwent sample collection from corneal ulcers by an ophthalmologist. The corneal scraping was performed using topical anesthesia with proxymetacaine (5 mg/ml) or, when necessary, narcosis in the operating room. We inoculated the collected material in solid (blood agar, chocolate agar and Sabouraud agar; Oxoid Ltd., Basingstoke, UK) and liquid (brain heart infusion and thioglycolate; Oxoid Ltd.) culture media[22-24]. The material was placed in a transport medium containing saline and sterile distilled water and was subsequently inoculated on plates with soy agar culture media (semi-defined), supplemented with 20 µl of inactivated *Escherichia coli* when an infection with the protozoan *Acanthamoeba* spp. was suspected[24]. In our laboratory, the routine practice of this specific culture began in 1987 with the identification and subsequent publication of the first cases of keratitis caused by *Acanthamoeba* spp. in Brazil[25]. Maintenance of the inoculated culture media, and isolation and identification of bacteria, fungi, and the protozoan *Acanthamoeba* spp. were performed according to standard microbiology procedures[22-24,26].

The assessment of bacterial susceptibility to antimicrobial agents commonly used in ophthalmology was performed by using the Kirby-Bauer agar-diffusion test according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS; Philadelphia, PA, USA) until 2004, and, subsequently, by the Clinical and Laboratory Standards Institute (CLSI; Philadelphia, PA, USA).

Categorical variables were analyzed using the chi-square test. Continuous variables were analyzed using Student's t test. A p value <0.1 was considered significant. Logistic regression was used with a p value <0.05 considered significant. MedCalc software version 11.6.1[27] (MedCalc, Ostend, Belgium) was used for the statistical analysis.

RESULTS

Between July 1975 and December 2010, a total of 859 patients with clinical suspicion of keratitis aged 0-18 years were assessed. From these patients, 346 eyes from 346 patients [197 (56.9%) boys] with positive cultures for bacteria, fungi and/or the protozoan *Acanthamoeba* spp. were included in the study.

The right eye was more commonly affected, in 184 (53.2%) of patients. The age ranged from 10 days to 18 years with a mean of 12 ± 4.7 years. We divided the patients according to age: there were 5 (1.4%) newborns (0-28 days), 47 (13.6%) infants (29 days to 2 years), 57 (16.5%) pre-school age children (3-6 years), 73 (21.1%) school children (7-12 years), and 164 (47.4%) adolescents (13-18 years). Thus, the most prevalent age group was the adolescents.

Topical medication was used at the time of sample collection in 42.8% of the patients. Antimicrobial drugs accounted for 71.6% of the topical medications used (Table 1). Major antecedents included trauma in 33.5% of patients, previous eye surgery in 24.9%, and the use of contact lenses in 18.2% (Table 1). Cancer was the major comorbidity found, in seven of the patients, accounting for 2% of the study patients.

Bacterial cultures were requested for all 346 patients and 309 (89.3%) tested positive. There were 317 requests for fungal cultures, with 14 (4.4%) testing positive and 97 requests for the protozoan *Acanthamoeba* spp. with 23 (23.7%) of these patients testing positive. Overall, 4% and 6.6% of all patients tested positive for fungi and *Acanthamoeba* spp., respectively.

A total of 344 microorganisms were isolated from the 309 positive bacterial cultures (71.8% Gram-positive and 28.2% Gram-negative). The most prevalent bacteria were coagulase-negative *Staphylococcus* (23.8%), *Staphylococcus aureus* (20.9%), and *Pseudomonas* spp. (14.2%) (Table 2). Polymicrobial cultures accounted for 9.5% of the positive cultures.

Table 1. Frequency of ocular antecedents among patients aged from 10 days to 18 years with keratitis

Eye history	Total (%)
Ocular antecedent	
Use of topical medications	148 (42.8)
Trauma	116 (33.5)
Prior eye surgery	86 (24.9)
Use of contact lenses	63 (18.2)
Conjunctivitis	24 (6.9)
Comorbidities	20 (5.8)
Congenital glaucoma	13 (3.8)
Keratitis	11 (3.2)
Keratoconus	8 (2.3)
Herpes	7 (2.0)
Atopy	3 (0.9)
Blepharitis	3 (0.9)
Endophthalmitis	2 (0.6)
Stye	2 (0.6)
Proptosis	2 (0.6)
Nodular degeneration Salzmann	1 (0.3)
Entropion	1 (0.3)
Microphthalmos	1 (0.3)
Trachoma	1 (0.3)

Table 2. Prevalence of bacteria isolated from keratitis samples among patients aged from 10 days to 18 years

Bacteria	Total (%)
Coagulase-negative *Staphylococcus*	82 (23.8)
Staphylococcus aureus	72 (20.9)
Pseudomonas spp.	49 (14.2)
Streptococcus pneumonia	31 (9.0)
Streptococcus viridans	28 (8.1)
Corynebacterium spp.	21 (6.1)
Moraxella spp.	16 (4.6)
Serratia spp.	10 (2.9)
Haemophilus spp.	8 (2.3)
Streptococcus spp.*	8 (2.3)
Unidentified Gram- bacilli	3 (0.9)
Unidentified Gram+ bacilli	3 (0.9)
Enterobacter spp.	3 (0.9)
Proteus spp.	3 (0.9)
Klebsiella spp.	2 (0.6)
Acinetobacter spp	1 (0.3)
Bacillus circulans	1 (0.3)
Citrobacter spp	1 (0.3)
Neisseria spp	1 (0.3)
Nocardia asteroides	1 (0.3)
Total	**344**

*= *Streptococcus* spp.: gamma-hemolytic (4), spp. (3) and beta-hemolytic (1).

Thirteen filamentous fungi and one yeast, *Rhodotorula rubra*, were identified. Filamentous fungi included *Fusarium solani* species complex (64.3%), *Aspergillus flavus* species complex, *Scedosporium apiospermum*, *Penicillium* spp. (7.1% each) and one unidentified.

All of the Gram-positive cocci were vancomycin-susceptible. Methicillin-susceptible *Staphylococcus* showed resistance lower than 10% to first generation cephalosporins, aminoglycosides, and fluoroquinolones, and resistance lower than 20% to trimethoprim-sulfamethoxazole. Methicillin-resistant *Staphylococcus* showed significant resistance to most antimicrobials apart from the most recent generations of fluoroquinolones. *Streptococcus pneumoniae* showed no resistance to penicillin.

Enterobacteriaceae showed full susceptibility to amikacin, ceftriaxone, ciprofloxacin, chloramphenicol, gentamicin, moxifloxacin, and ofloxacin, whereas tobramycin and trimethoprim-sulfamethoxazole showed 5.6% and 12.5% resistance, respectively. *Pseudomonas aeruginosa* showed 100% susceptibility to amikacin, ciprofloxacin, and ofloxacin and resistance lower than 6% to gentamicin, tobramycin, and polymyxin B.

We analyzed the annual incidence of cases of keratitis in children and adolescents and the respective identification of microorganisms over the study period. The percentage of fungal infections remained stable throughout the study period, with positivity ranging from 1.8% to 16.6%. The prevalence of infections with *Acanthamoeba* spp. ranged from 1.8% to 11.1%. Bacterial infections were always the most common, with the prevalence in cultures ranging from 10% to 100%.

Univariate analysis comparing patients with bacterial and non-bacterial keratitis showed the following significant variables for non-bacterial keratitis: age (p=0.001), the use of antimicrobial drugs (p=0.02), and non-chemical or physical burns (p=0.004). In the multivariate analysis, age (odds ratio [OR] 1.19; 95% confidence interval [C] I 1.09-1.31), use of antimicrobial drugs (OR 2.50; 95%CI 1.19-5.23), and physical burn injuries (OR 17.29; 95%CI 2.40-124.57) were associated with non-bacterial keratitis (fungal or *Acanthamoeba* spp.) (Table 3).

Table 3. Univariate and multivariate analyses comparing bacterial versus non-bacterial keratitis among patients aged from 10 days to 18 years

	Bacterial keratitis (N=309)	Non-bacterial keratitis (N=37)	p-value	Odds ratio	95%CI	p-value
Variables						
Age, years	10.0 ± 6.0	14.6 ± 3.4	**0.001**	1.19	1.09 - 1.31	**0.0002**
Male sex	179 (57.9)	18 (48.6)	0.370			
Affected right eye	166 (53.7)	18 (48.6)	0.680			
Eye history						
Atopy	3 (1.0)	0 (0.0)	0.740			
Blepharitis	3 (1.0)	0 (0.0)	0.740			
Keratitis	8 (2.6)	1 (2.7)	0.610			
Keratoconus	8 (2.6)	1 (2.7)	0.610			
Conjunctivitis	23 (7.4)	1 (2.7)	0.470			
Endophthalmitis	2 (0.6)	0 (0.0)	0.510			
Congenital glaucoma	13 (4.2)	0 (0.0)	0.420			
Herpes	6 (2.0)	1 (2.7)	0.760			
Stye	2 (0.6)	0 (0.0)	0.510			
Proptosis	2 (0.6)	0 (0.0)	0.510			
Antimicrobial use	88 (28.5)	18 (48.6)	**0.020**	2.50	1.19 - 5.23	**0.0100**
Injury						
Vegetable	41 (13.2)	0 (0.0)	**0.040**			
Mechanical	15 (4.8)	1 (2.7)	0.860			
Metal	15 (4.8)	3 (8.1)	0.650			
Chemical burn	3 (1.0)	0 (0.0)	0.740			
Non-chemical burn	2 (0.6)	3 (8.1)	**0.004**	17.29	2.40 - 124.57	**0.0050**
Prior eye surgery						
Corneal transplantation	36 (11.6)	7 (18.9)	0.320			
Postoperative complications	15 (4.8)	1 (2.7)	0.510			
Removal of corneal foreign body	7 (2.3)	1 (2.7)	0.680			
Suture	4 (1.3)	2 (5.4)	0.250			
Eyelid correction	2 (0.6)	0 (0.0)	0.510			
Tumor excision	2 (0.6)	0 (0.0)	0.510			
Coating	2 (0.6)	0 (0.0)	0.510			
Blepharorrhaphy	2 (0.6)	0 (0.0)	0.510			
Antiglaucoma tube insertion	2 (0.6)	0 (0.0)	0.510			
Type of contact lenses						
Soft	29 (9.4)	7 (18.9)	0.130			
Rigid	6 (1.9)	0 (0.0)	0.850			
Therapeutic	3 (1.0)	0 (0.0)	0.740			
Comorbidities						
Cancer	7 (2.3)	0 (0.0)	0.760			
Chemo or radiation therapy	2 (0.6)	0 (0.0)	0.510			

Data are presented as mean ± standard deviation or n (%).

In univariate analysis that compared patients according to whether the keratitis was caused by Gram-negative or by Gram-positive bacteria, age (p=0.01) was found to be statistically significant. Indeed, logistic regression showed age (OR 1.06; 95%CI 1.01-1.11) to be a risk factor for keratitis caused by Gram-negative bacteria in the (Table 4).

DISCUSSION

Microbial keratitis and its sequelae are important causes of ocular morbidity and blindness in developing countries. Microbial keratitis is characterized by inflammation of the cornea, usually with stromal infiltration, and is considered an ophthalmic emergency that requires immediate attention. Keratitis can progress rapidly with corneal destruction that causes permanent visual dysfunction.

In the literature, childhood is defined as the period of growth from birth to puberty. However, the definition of the limits of chronological adolescence is controversial. For the World Health Organization, the age is between 10 and 19 years whereas for the United Nations Organization, it is between 15 and 24 years. In Brazil, the Child and Adolescent Statute, law 8.069 of 1990, considers a child to be a person under 12 years old and defines adolescence as the age range 12-18 years[28].

Childhood keratitis differs from adult keratitis in several respects, including possible reluctance of the child to undergo the clinical examination and sample collection, and the information-gathering regarding triggering factors and inflammation, which is apparently more intense in children[3,15]. Among teenagers, contact lenses are becoming increasingly popular as a refractive error correction device, for esthetic reasons. The association between the use of contact lenses and infectious ulcers is well established.

During the period analyzed, 10.1% of keratitis cases occurred in the age group up to 18 years, mostly among male adolescents. These data corroborate previous studies in which keratitis in children and adolescents accounted for 11% to 13.1% of all ophthalmologic visits[3,8].

Our study found that age, the use of topical antimicrobial drugs, and prior physical burns were associated with fungal and *Acanthamoeba* spp. keratitis. Age was also associated with keratitis caused by Gram-negative bacteria. As far as we know, no previous study has analyzed this subject.

In our series, the use of topical medication was present in 42.8% of patients at the time of laboratory evaluation. Other authors have already indicated the use of empirical medication at the time of sample collection in 48% to 67.1% of patients with clinical suspicion

Table 4. Univariate and multivariate analyses comparing keratitis caused by Gram-negative bacteria with that caused by Gram-positive bacteria among patients aged from 10 days to 18 years

	Gram-positive bacteria (N=213)	Gram-negative bacteria (N=86)	p-value	Odds ratio	95%IC	p-value
Variables						
Age, years	9.9 ± 5.5	11.6 ± 5.2	**0.01**	1.06	1.01-1.11	**0.01**
Male sex	118 (55.4)	55 (63.9)	0.22			
Right eye affected	118 (55.4)	41 (47.6)	0.28			
Eye history						
Atopy	3 (1.4)	0 (0.0)	0.64			
Blepharitis	2 (0.9)	1 (1.2)	0.64			
Keratitis	7 (3.3)	1 (1.2)	0.53			
Keratoconus	5 (2.3)	2 (2.3)	0.68			
Conjunctivitis	17 (8.0)	6 (7.0)	0.96			
Congenital glaucoma	11 (5.2)	1 (1.2)	0.20			
Herpes	6 (2.8)	0 (0.0)	0.26			
Stye	2 (0.9)	0 (0.0)	0.91			
Use of antimicrobial	67 (31.5)	19 (22.1)	0.14			
Injury						
Vegetable	30 (14.1)	11 (12.8)	0.91			
Mechanical	10 (4.7)	4 (4.6)	0.77			
Metal	7 (3.3)	7 (8.1)	0.13			
Non-chemical burn	3 (1.4)	0 (0.0)	0.64			
Chemical burn	0 (00.0)	2 (2.3)	0.15			
Prior eye surgery						
Corneal transplantation	24 (11.3)	11 (12.8)	0.86			
Postoperative complications	15 (7.0)	0 (0.0)	**0.02**			
Removal of corneal foreign body	6 (2.8)	1 (1.2)	0.66			
Suture	2 (0.9)	0 (0.0)	0.91			
Comorbidity						
Tumor	5 (2.3)	2 (2.3)	0.68			
Type of contact lenses						
Soft	25 (11.7)	4 (4.6)	0.10			
Rigid	3 (1.4)	2 (2.3)	0.95			
Therapeutic	1 (0.5)	2 (2.3)	0.41			

Data are presented as mean ± standard deviation or n (%).

of keratitis[3,9,16]. As they are already widespread, the use of antibiotics can inhibit bacterial growth, favoring the appearance of other microorganisms. This factor was statistically significant in the group of patients with non-bacterial keratitis and may be considered as a predisposing factor.

A history of trauma was the most common antecedent found in our study as cause of keratitis in the age range studied, affecting 33.5% of patients. Trauma has been reported as the major risk factor for keratitis in several published studies, accounting for 21.2% to 58.8% of cases[8-18,29].

In our study, 18.2% of patients had a history of using contact lenses. Soft lenses were the most commonly used type. More than 90% of patients with a positive culture for *Acanthamoeba* spp. were contact lens users and all were adolescents. As has been said, the association between the use of contact lenses and infectious ulcers is well established. Several authors have reported the use of contact lenses, especially orthokeratology, as a predisposing factor for keratitis in children and adolescents[3,8-10,14,17,19,20,30]. The use of this type of lens is not common in Brazil.

Comorbidities were present in 5.8% of the study patients; this was lower than rates reported in previous studies[3,8-14]. Cancer was the main comorbidity, present in 2% of our records. This percentage was lower than the rate of 3.8% reported by Onakpoya et al.[11]. Other ocular antecedents found in our study have been reported by several authors, citing similar percentages[3,10,11,19].

The prevalence of microorganisms responsible for microbial keratitis in children and adolescents varies in different regions of the world[3]. Microbial cultures were positive in 40.3% of the study sample, whereas in the literature positivity has ranged from 33% to 87%[3,8-10,12-18,20,29]. Polymicrobial isolates accounted for 9.5% of positive cultures in our study. In previous studies, the positivity of polymicrobial cultures has varied from 6.9% to 27%[3,8-10,12,14]. As observed in other studies, the inherent difficulty in getting corneal samples from patients, the use of antimicrobials, improper corneal sampling, and microorganisms with slow growth on culture media may account for the low positivity rate observed in our study[3,15].

The presence of Gram-positive bacteria in keratitis in this study was 71.8%, similar to the level previously reported elsewhere[3,8,9,12-17,19,20,29].

Of the identified etiologic agents, the most common was coagulase-negative *Staphylococcus*, which was isolated from 23.8% patients; this was followed by *Staphylococcus aureus* (20.9%) and *Pseudomonas* spp. (14.2%). These results were similar to those observed in the elderly population in a previous study conducted in our institution[2].

Methicillin-resistant *Staphylococcus* spp. showed a trend of resistance to most antimicrobials, something that has also been observed by other authors[15,19,20,29], whereas coagulase-negative *Staphylococcus*, *Streptococcus* spp., and enterobacteria showed good susceptibility to the majority of antimicrobial drugs tested. *Streptococcus pneumoniae* strains showed no resistance to penicillin. *Pseudomonas aeruginosa* strains showed no resistance to ciprofloxacin and low resistance to polymyxin B, gentamicin, and tobramycin, in contrast to the study by Moreno-Andrade et al., who reported 100% resistance to this antimicrobial[29].

The positivity of fungal infection among children and adolescents was 4% in our study, whereas the positivity for fungal infection in previously published studies has ranged from 4% to 48.7%[3,8-10,12-14,16,17,19]. *Fusarium solani* complex was the most commonly isolated fungus, as was the case in other previous studies[3,10,17].

Keratitis caused by the protozoan *Acanthamoeba* spp. showed 6.6% positivity in the present study, which again is similar to previous studies[3,9,12,19]. It is noteworthy that all patients with positive culture for *Acanthamoeba* spp. were adolescents and that 91.3% had a history of using contact lenses. It is likely that increasing the frequency of use of contact lenses by teenagers increases the risk of adolescents developing corneal ulcers by *Acanthamoeba* spp.

In conclusion, we found that the risk factors for infectious keratitis in children and adolescents were noticeably the same as those observed in adults. We found that age, the use of antimicrobials, and injuries from physical burns were predisposing factors for non-bacterial keratitis. Age was associated with Gram-negative keratitis.

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