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Corneal ulcer: analysis of isolated bacteria and antibiotics sensitivity in the urban region of Attica, Greece

Abstract

Introduction: The presence of a corneal ulcer stresses the ophthalmologist as it can be vision threatening. It may very often be difficult to treat as the antibiotics used in current practice often seem inefficient. The aim of the current study is to present the sensitivity and resistance of the commonly used topical antibiotics in patients with corneal ulcers.

Materials and methods: Smears from patients with suspected non sterile corneal ulcers (CU) and corneal ulcers, the contact lenses and their disinfection solutions in infected individuals (CL) were taken. Identification of the cultured microorganisms and an antibiogram, were performed automatically (MICROSCAN AUTO SCAN (DADE BEHRING) and API system).

Results: In total 82 smears were taken. 64,15% of the smears had positive cultures for bacteria (75% in the CL group and 53.6% in the CU group). In the CU group Gram+ (G+) bacteria were isolated more frequently whereas in the CL group this was true for Gram-(G-) bacteria. G-bacteria (besides *pseudomonas*) were notably resistant to amino glycosides and quinolones whereas Gram+ bacteria showed great sensitivity in most antibiotics.

Conclusion: 'Blind' use of amino glycosides or other antibiotics for CU treatment needs to be re-evaluated. Choice for the adequate treatment should always be proposed based on the antibiogram and for this close co-operation of ophthalmologists with the microbiology laboratory is needed. Also, CL users need to be instructed for the correct use and disinfection of their CLs.

Keywords: microorganism, infection, culture, contact lens, smear

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Introduction

Corneal ulcer is a serious vision threatening inflammation that may even lead to the loss of an eye. It can be caused by several bacteria agglutinating to the corneal epithelium that has lost its integrity after trauma. Very few bacteria (*listeria, haemophilus, neisseria, gonorrhea* and *corynebacterium diphtheriae*) may in grow the intact corneal epithelium and cause an infective ulcer.[1-5] Inappropriate contact lens use is often incriminated, as 62,7% of the young corneal ulcer patients are contact lens users. Other factors that may favor the creation of an ulcer include: older age,[2,4,6] generally pathologic ophthalmic surface,[1,2,4,6-10] chronic steroid use (28,6%),[4,9] systemic disease (36.4%),[4,8-10] chronic blepharitis (20,8%),[9] previous ophthalmic surgery (33,8%)[1,2,4,6,8,9] and herpetic keratitis (28,85%).[2,4,9] Prognosis is worse in older patients, in diabetic patients and in Gram-bacterial infection. Adverse prognostic factors includes: presence of medium and large sized ulcers, hypopyon, a negative culture a dramatic loss of the visual acuity.[2]

The initial therapy usually requires broad spectrum antibiotics but their reckless use in everyday practice has lead either to treatment failure or to the development of resistant bacteria that make ophthalmic infection quite challenging to treat.[11,12] The correct treatment of an infectious corneal ulcer requires topical antibiotics based on the culture result. Several authors describe the bacteria isolated and their resistance to the antibiotics used.[1,2,4,6-12] The bacteria responsible for corneal ulcers may vary according to the patient's origin, climate, and regional conditions.[9,13] Bacterial resistance on the other hand depends on the antibiotics applied and their rational use.[9,13] For this reason a corneal ulcer culture and antibiogram for the isolated bacteria seem indispensable. The aim of the current study is to investigate, in the urban Greek population of Attica, the bacterial strains isolated in cultures taken from corneal ulcers, contact lenses and disinfecting solutions and to evaluate the resistance or sensitivity *in vivo* as seen in the antibiograms.

Materials and methods

This is a retrospective study investigating and analyzing the results of corneal ulcer cultures in smears taken from corneal ulcers as well as, contact lenses and the disinfection solution in individuals infected that were contact lens users. All data were collected from the microbiology laboratory registry of the Patission Hospital during a period of 30 months. The research followed the tenets of the Declaration of Helsinki. A corneal ulcer culture was performed to every patient coming to the Emercencies Room of the Outpatient Ophthalmology Department presenting a possible infectious corneal ulcer of any etiology but also with ulcers under treatment but no epithelialization. When the patient was wearing contact lenses the lenses were sent for culture together with the disinfecting solution used.

The culture was taken during the patient's first visit, on the slit lamp using a swab from the center and the borders of the ulcer (in large ulcers) or the whole surface of the ulcer in small lesions. The swab was manipulated in a circular spiral way avoiding the lids and the eyelashes without the use of anesthetic and despite the pain

caused that was well tolerated by all patients following that, the swab was immersed into the cultivation medium and transported to the microbiology laboratory. Inoculation of the samples was made in a blood agar, MacConkey, Chocolate, Chapman and Sabouraud media. Identification and susceptibility testing were done with the automated system MICROSCAN AUTO SCAN (DADE BEHRING) and when necessary API system was used for the identification and disk diffusion method of antibiotics in agar for the antibiogram. The mean time between the collection and the culture was 15 minutes whereas the mean time between the culture and the antibiogram was 2 days.

A combined treatment of Quinolones drops and Ceftazidime and Amikacin mixed solution in drops were administered while waiting for the antibiogram results. If the patient was already in a well responding treatment, this was not changed even if the antibiogram showed resistance for the specific regimen. When fungi were suspected Fluconazole drops and Itraconazole per OS were also given. The data collected, included the number and type of bacteria developed in the collected specimens as well as their sensitivity in the antibiotics indicated by the antibiogram.

Results

A total of 82 smears were examined during a period of 30 months. 42 of the smears were collected directly from the surface of corneal ulcers whereas the remaining 40 samples were from the contact lenses and their disinfection solutions (in some individual's one smear per eye). The majority of the samples were taken from females (47 women and 26 men). Fifty two (64.15%) cultures were positive for bacteria (53.6% (22) in CU and 75% (30) in CL). A single bacteria was isolated in 27 of the 52 (51.9%) positive cultures and in the remaining 25 (48%) 2-4 bacteria were found. More specifically in the CL group a single bacteria was found in 14(46.6%) and multiple bacteria in 16 (53.3%) samples whereas in the CU group a single bacteria was isolated in 13 (59%) cultures and multiple bacteria in 9 (41%).

Bacteria isolated

Contact lenses: Table 1 indicates the bacteria isolated in this group as well as the number of the strains for each bacteria.

Table 1 The number of isolated bacteria in the CL group and their corresponding strains. We may see a predominance of *Gram- bacteria*

Isolated Bacteria in CL.	
Isolated Bacteria	**Strain No**
AerobicG-	18
(PAeruginosa)	-10
EnterobacterG-	17
(Serratiamarcescens)	-7
G+ Cocci	6
(Streptococcus SPP	-4
Staphylococcus CNS	-1
Enterococcus faecalis)	-1
G+ bacteria	2
(Corynobacterium spp	-1
Bacillus spp	-1
Moraxella species	1
Acinetobacter Lwoffii	1
Neisseria spp	1
*Fungi**	6

*Present in multi microbial cultures

Corneal ulcers: Table 2 indicates the more commonly isolated bacteria for this group and the number of their strains.

Table 2 The isolated bacteria in the CU group showing a predominance of the CNS

Isolated Bacteria on CU.	
Isolated Bacteria	**Number of Strains**
CNS	13
Corynobacterium	3
Micrococcus	2
Bacillus	1
Candida	2
Streptococcus	8
Staphylococcus aureus	2
P Aeruginosa	2
Serratia Marcescens	1

Sensitivity of the isolated bacteria to antibiotics

Gram– *P Aeruginosa* that was more commonly isolated was sensitive in Ciprofloxacin (100%), Levofloxacin (100%), Chloramphenicol (100%), Ceftazidime (100%), Piperacillin (100%), Amikacin (80%), Tobramycin (80%), Gentamicin (70%,) Aztreonam (70%), Ceftriaxone (10%). The remaining aerobic strains, besides pseudomonas were notably resistant to amino glycosides and quinolones (Table 3). Sensitivity of the remaining Enterobacter and the isolated strains of *Serratia marcescens* are presented in Table 4.

Table 3 The majority of the *Gram- bacteria* show resistancy to the commonly used antibiotic

Aerobic *Gram- Bacteria* Resistance (exc *P Aeruginosa*).		
Antibiotic	**Serratia *Marcescens***	**Remaining *Enterobacters***
Amikacin	100%	100%
Levofloxacin	100%	100%
Ceftazidime	100%	100%
Tetracycline	57%	88%
Cefuroxime	14%	53%
Ampicillin	14%	35%
Ciprofloxacin	100%	100%
Cephalothin	28%	63%
Tobramycin	100%	100%
Gentamycin	86%	94%
Cefoxitin	42%	53%
Amoxicillin-Clav	14%	41%
Cefazolin	28%	63%
Cefotaxime	86%	63%
Chloramphenicol	100%	100%
Aztreonam	86%	63%

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Table 4 *Serratia marcescens* and the remaining *enterobacter* show a great sensitivity to Amikacin, Levofloxacin and Caftazidime

Sensitivity of Serratia *Marcescens* and the Remaining *Enterobacter*		
Antibiotic	**Serratia *Marcescens***	**Remaining *Enterobacters***
Amikacin	100%	100%
Levofloxacin	100%	100%
Ceftazidime	100%	100%
Tetracycline	57%	88%
Cefuroxime	14%	53%
Ampicillin	14%	35%
Ciprofloxacin	100%	100%
Cephalothin	28%	63%
Tobramycin	100%	100%
Gentamycin	86%	94%
Cefoxitin	42%	53%
Amoxicillin-Clav	14%	41%
Cefazolin	28%	63%
Cefotaxime	86%	63%
Chloramphenicol	100%	100%
Aztreonam	86%	63%

Gram+ The most commonly strain was CNS (*coagulase negative staphylococci*) that was sensitive to Vancomycine (100%), Ciprofloxacin (92%), Chloramphenicol (92%), Clindamycin (92%), Tetracycline (84%), Gentamycin (80%), Fucidic Acid (69%), Oxacillin (61%), Cefotaxime (61%), Cefepime (61%), Cefuroxime (61%), Meropemen (61%), Imipenem (61%), Penicillin (38%). *Fungi* were isolated in 8 cultures that were already positive for other bacteria (8 positive, 6 cultures in CL).

Discussion

The effective treatment of infectious corneal ulcers is based primarily on the identification of the responsible agent followed by the correct choice of the antibiotic based on an antibiogram, its appropriate dosage and frequency of administration. The main questions that arise directly after the diagnosis of a corneal ulcer are the nature of the responsible bacteria and the appropriate treatment to be administered. According to guidelines, for a corneal ulcer culture, the diameter of the lesion needs to be > 2 mm to have a positive culture in 52.5%-73%.[1,5-7,11-15] In our study contrariwise to previous studies, cultures were taken in every patient presented to the emergencies with a corneal ulcer, regardless of the size of the lesion or whether the patient was already under treatment or not and this is the only limitation of our study. This may be the reason of our report reaching 64.15% of positive cultures.

The patient was placed under treatment while waiting for the culture results, taken into account the possible causes, the location and the characteristics of the lesion. Unfortunately the frequent irrational use of topical and systemic antibiotics has lead to microorganism's resistant to the more frequently used antibiotics such as Quinolones and Amino glycosides.[9,16-18] The fact that newer antibiotics as Quinolones became resistant, confirms that topical instillation may lead to resistancy. This makes treatment even more challenging, and leads pharmaceutical companies to the discovery of newer and more aggressive antibiotics.[11,12,19] The antibiograms of the current study confirm that bacterial sensitivity is really limited to the frequently used antibiotics. In corneal ulcers the most common Gram+ bacterium found was CNS that remained sensitive to Vancomycin, a hospital administered medication.[1,12,18] Its sensitivity to other antibiotics was more limited.

Amino glycoside resistancy is most puzzling since it was the treatment of choice in everyday clinical practice. Previous studies regarding Quinolones and Amino glycosides show diversity. Afshari et al.,[11] report increased resistance in Quinolones but not in Gentamycin while in the paper of Azevedo Gayoso et al.,[19] there is a clear decrease of *in vitro* sensitivity in Methicilline, Tobramycin and Gentamycin whereas it remains stable in Ofloxacine and Ciprofloxacin. In contact lens users *P aeruginosa* was the most frequently isolated Gram negative strain. It is generally regarded as a problematic strain since it is highly resistant toward various antimicrobial agents. In our study it found sensitive to Quinolones, Ceftazidime and less in the Amino glycosides. Those results differ from those presented by Mah-Sadora et al.,[3] that report an *in vivo* resistance of Quinolones despite the *in vitro* sensitivity. Possible diversity between *in vitro* and *in vivo* sensitivity of bacteria must be kept in mind when dealing with corneal ulcer management.[15] Discrepancy between studies concerning antibiotic sensitivity could be due to regional differences and more specifically to geographic location, climate and clinical practice use.[16-18]

Serratia presented sensitivity to Quinolones and Amikacin.[15,20] For the remaining Gram- bacteria quinolones and Ceftazidime were sensitive while Gentamicin was very resistant. Fortunately, Chloramphenicol revealed to be sensitive for *P aeruginosa* and *Serratia*.[21,22] Other bacteria, such as *A Xyloxosidans* found only in CL multi microbial cultures and not in CU cultures, were very resistant to classic antibiotics. This bacterium was responsible for opportunistic infection in immunocompromised patients using for a long time cortisone drops.[1] Its isolation in the CL disinfection solution is very important since it indicates that *A Xyloxosidans* eventually cause an ulcer in healthy individuals. Also, the fact that it is resistant to a great number of antibiotics makes treatment more challenging. These findings along with the isolation of fungi to the disinfection solution indicate bad sanitary conditions of the CL users and the need for serious prevention measures.

A very interesting finding was the multi bacterial cultures representing 53% of the CL cultures and 41% of the CU cultures. These results are surprisingly very elevated compared to those in previous studies that reported values varying between 13-21%.[1,9,11,13,14,15,20] Climatic differences and bad CL maintenance could be responsible for this important variation. Although the technological improvements regarding CL materials and disinfection solutions have been really important lately, ulcerative keratitis remains the major concern of CL users since this is the predisposing factor for the development of a corneal ulcer in young and middle aged individuals in the developed countries as the users increase daily worldwide. (26000000 users in1997 à 125000000 in 2007).[2,4] The current study confirms that Gentamycin and Tobramycin, vastly used in clinical practice by the ophthalmologist and other health professionals, are not sufficiently

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sensitive and should be used with caution. Quinolones and 3rd generation Cephalosporins are very effective for most of the Gram- bacteria and *Pseudomonas*. Also Chloramphenicol exhibits excellent performance whereas Vancomycin is the only one with complete sensibility to CNS.

Another factor affecting the ophthalmologist's decision especially in patients remaining in long term ophthalmic drop treatment is systemic toxicity. Toxicity is concentration dependent and thus, only patients with chronic disease could be eventually affected. Chloramphenicol is the only topical antibiotic treatment incriminated for possible systemic risks (hepatitis, bone marrow suppression). It should be noted that the minimum reported total topical dose of chloramphenicol proposed to be associated with marrow toxicity is 30 mg and the minimum associated duration of exposure is 18 days.[23,24] It is also evident that special recommendations should be made to CL users since mixed pathogenic flora of resistant microorganisms may develop. Repeating the medical instructions of contact lens maintenance and disinfection may be necessary as a preventive measurement of corneal ulceration.[3] Finally, a close collaboration with the microbiology laboratory is advised for the stratification of the medical treatment.

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None.

Conflicts of interest

Authors declare that there is no conflict of interest.

References

1. Green M, Apel A, Stapleton F. Risk Factors and Causative Organisms in Microbial Keratitis. *Cornea*. 2008;27(1):22–27.
2. Ly CN, Pham JN, Badenoch PR, et al. Bacteria commonly isolated from keratitis specimen retain antibiotic susceptibility to fluoroquinolones and gentamicin plus cephalothin. *Clin Experiment Ophthalmol*. 2006;34(1):3–5.
3. Mah-Sadorra JH, Yavuz SG, Najjar DM, et al. Trends in Contact Lens Related Corneal Ulcer. *Cornea*. 2005;24(1):51–58.
4. Bourcier T, Thomas F, Borderie V, et al. Bacterial Keratitis: predisposing factors, clinical and microbiological review of 300 cases. *Br J Ophthalmol*. 2003;87(7):834–838.
5. Ibrahim YW, Boase DL, Cree IA. Epidemiological characteristics, predisposing factors and microbiological profiles of infectious corneal ulcers: the Portsmouth corneal ulcer study. *Br J Ophthalmol*. 2009;93(10):1319–1324.
6. Ancele E, Lequeux L, Fournie P, et al. Severe bacterial keratitis. A clinical, epidemiologic and microbiologic study. *J Fr Ophthalmol*. 2009;32(8):558–565.
7. Cruciani F, Cuozzo G, Di Pillo S, et al. Predisposing factors, clinical and microbiological aspects of bacterial keratitis: a clinical study. *Clin Ter*. 2009;160(3):207–210.
8. Saeed A, D'Arcy F, Stack J, et al. Risk factors, microbiological findings, and clinical outcomes in cases of microbial keratitis admitted to a tertiary referral center in Ireland. *Cornea*. 2009;28(3):285–292.
9. van der Meulen IJ, van Rooij J, Nieuwendaal CP, et al. Age-related Risk Factors, Culture Outcomes, and Prognosis in Patient Admitted With Infectious Keratitis to two Dutch Tertiary Referral Centers. *Cornea*. 2008;27(5):539–544.
10. Jhanji V, Constantinou M, Taylor HR, et al. Microbiological and Clinical Profile of Patients with Microbial Keratitis Residing in Nursing Homes. *Br J Ophthalmol*. 2009;93(12):1639–1642.
11. Afshari NA, Ma JJ, Duncan SM, et al. Trends in resistance to ciprofloxacin, cefazolin, and gentamicin in the treatment of bacterial keratitis. *J Ocul Pharmacol Ther*. 2008;24(2):217–223.
12. Wong RL, Gangwani RA, Yu LW, et al. New treatments for bacterial keratitis. *J Ophthalmol*. 2012;2012:831502.
13. Mandour SS, Marey HM, Farahat HG. Resistant Microbial Keratitis in South Nile Delta, Egypt: Influence of Regional Risk Factors. *Semin Ophthalmol*. 2014;14:1–7.
14. Yeh DL, Stinnett SS, Afshari NA. Analysis of bacterial cultures in infectious keratitis, 1997 to 2004. *Am L Ophthalmol*. 2006;142(6):1066–1068.
15. Mah-Sadorra JH, Najjar DM, Rapuano CJ, et al. Serratia corneal ulcers: A retrospective clinical study. *Cornea*. 2005;24(7):793–800.
16. Tokman HB, İskeleli G, Dalar ZG, et al. Prevalence and antimicrobial susceptibilities of anaerobic bacteria isolated from perforated corneal ulcers by culture and multiplex PCR: an evaluation in cases with keratitis and endophthalmitis. *Clin Lab*. 2014;60(11):1879–1886.
17. Ray KJ, Prajna L, Srinivasan M, et al. Fluoroquinolone treatment and susceptibility of isolates from bacterial keratitis. *JAMA Ophthalmol*. 2013;131(3):310–313.
18. Ni N, Nam EM, Hammersmith KM, et al. Seasonal, geographic, and antimicrobial resistance patterns in microbial keratitis: 4-year experience in eastern Pennsylvania. *Cornea*. 2015;34(3):296–302.
19. Azevedo Gayoso Mde F, Oliveira AD, d'Azevedo PA, et al. *In vitro* antimicrobial susceptibility of coagulase negative staphylococcal ocular isolates. *Arg Bras Oftalmol*. 2007;70(6): 924–928.
20. Konda N, Motukupally SR, Garg P, et al. Microbial analyses of contact lens-associated microbial keratitis. *Optom Vis Sci*. 2014;91(1):47–53.
21. Hoddenbach JG, Boekhoorn SS, Wubbels R, et al. Clinical presentation and morbidity of contact lens-associated microbial keratitis: a retrospective study. *Graefes Arch Clin Exp Ophthalmol*. 2014;252(2):299–306.
22. Prokosch V, Gatzioufas Z, Thanos S, et al. Microbiological findings and predisposing risk factors in corneal ulcers. *Graefes Arch Clin Exp Ophthalmol*. 2012;250(3):369–374.
23. Doshi B, Sarkar S. Topical administration of chloramphenicol can induce acute hepatitis. *BMJ*. 2009;338:b1699.
24. Phillips CI. Risk of systemic toxicity from topical ophthalmic chloramphenicol. *Scott Med J*. 2008;53(3):54–55.