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**Short Communication**

# Infectious Keratitis: Microbiological Review of 297 Cases

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**SUMMARY:** Infectious keratitis is a serious ocular infection that can lead to loss of vision. The aim of this study was to investigate the microbiological characteristics of this infection at the University Hospital of Guadalajara (Spain). We retrospectively reviewed all cases diagnosed between January 2010 and December 2016. During the 7-year study period, 297 corneal scrapes corresponding to 298 patients were performed. Antibiotic treatment prior to the culture was administered in 59 cases (19.9%). Contact lens wear was the most common risk factor (33.2%). Bacterial keratitis accounted for 64.6% of cases, viral keratitis for 3.4%, and fungal keratitis for 1%. A total of 241 bacterial strains were identified. Gram-positive isolates represented 87.1%, and gram-negative 12.7%. Coagulase-negative *Staphylococcus* strains were the most common microorganisms isolated (30.3%). When gram-positive microorganisms were analyzed, the sensitivity prevalence rates for vancomycin (VCM), levofloxacin, gentamicin (GM), and tobramycin (TO) were 99.4%, 84.6%, 87.9%, and 88.3%, respectively. For the gram-negative organisms, the sensitivity prevalence rates for ceftazidime, ciprofloxacin, GM, and TO were 83.3%, 93.5%, 96.3%, and 100%, respectively. Our study revealed strong predominance of gram-positive microorganisms. We suggest empirically treating bacterial keratitis originating in our area with VCM and TO, especially severe bacterial keratitis and pretreated cases in the community without a clinical response.

Infectious keratitis is a serious ocular infection that can lead to corneal opacity and loss of vision. Among risk factors, contact lens wear is the most common cause of corneal infection in developed countries (1). Because of potential rapid progression of the disease and devastating outcomes for vision, early and effective treatment is recommended. Initial management should begin with sample collection for culture followed by immediate antibiotic treatment (2). In the present study, we review all infectious-keratitis cases diagnosed at the University Hospital of Guadalajara (Spain) over a 7 year-period, to determine the causative organisms and antibiotic susceptibility patterns to better guide standard treatments.

We retrospectively reviewed the microbiological results on all infectious keratitis cases that were diagnosed at the University Hospital of Guadalajara (Spain), a 400-bed teaching hospital, between January 2010 and December 2016. Suspected infectious keratitis was defined as a corneal epithelial defect with a stromal infiltrate. Hospital records were reviewed to document demographic data, risk factors, and microbiological results. The risk factors studied were contact lens wear, blepharitis, trauma, immunosuppression, and previous surgical treatment. Polymicrobial keratitis was defined as a case where 2 or more types of pathogens were identified in corneal samples. The corneal scrapings were performed without a topical anesthetic agent under direct visualization using a slit lamp biomicroscope. The corneal samples were obtained using sterile surgical spears (Ivalon®, Fabco, New London, CT, USA) and sterile gloves. The patients who did not undergo corneal scraping were excluded from the study. Four samples were collected from each patient for bacterial culture. An additional sample was taken for fungal culture. The detection of fungi was performed for all patients. Gram staining was not performed because the amount of each sample was very low. The processing of the cultures was performed by standard procedures. Identification of isolates was performed by common biochemical tests: the API system (bioMérieux, Marcy l'Etoile, France) or the Vitek II system (bioMérieux). Antibiotic susceptibility testing was conducted using susceptibility cards by Vitek (bioMérieux) or the E-Test method (AB Biodisk, Solna, Sweden), according to Clinical and Laboratory Standards Institute (CLSI) interpretative criteria (3). When there was a suspicion of herpetic infection, a corneal sample was taken to perform PCR (RealCycler® Monotest HSVTVA v.4, Progenie Molecular, Valencia, Spain). The criteria for a suspected herpetic infection were the following: a recent history of a herpetic infection, stromal opacification, a dendritic pattern, keratic precipitates, and necrotizing stromal keratitis (4). Statistical analyses were performed using the QuickCalcs function of the GraphPad web tool (GraphPad Software, Inc., La Jolla, CA, USA, 2017). To determine whether there were statistically significant differences between extreme frequencies, Fisher's exact test was applied with a $2 \times 2$ contingency table. A value of $p < 0.05$ was assumed to denote statistical significance.

During the 7-year study period, 297 corneal scrapes corresponding to 298 patients were taken. Age of the

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patients was 49.3 ± 22.9 years (mean ± SD; range: 3 weeks to 98 years). The ratio of men to women was 1.0:1.15. Only one case was bilateral. Antibiotic treatment prior to the culture was documented in 59 cases (19.9%). The ocular predisposing factors under study were present in 63.7% of the cases. Contact lens wear was the most common risk factor (33.2%), followed by blepharitis (19.7%), trauma (13.2%), immunosuppression (6.8%), and previous surgical treatment (2.7%). There was no significant statistical difference between contact lens wear and the rest of risk factors ($p > 0.05$). Microbiological results were positive in 69% of cases. PCR analysis for the herpes virus was performed on 26 samples. Bacterial keratitis accounted for 64.6% of the cases, viral keratitis for 3.4%, and fungal keratitis for the remaining 1%. The etiology was unknown in 31% of the cases. When sterile cultures were analyzed, 55.5% of the patients had a history of antibiotic treatment. The prevalence of bacterial keratitis remained stable during the study period ($p = 0.078$). Trends for fungal and viral infections were not significant. The isolated microorganisms are shown in Table 1. A total of 241 bacterial strains were identified. Two or more bacterial taxa were isolated from 31 samples (10.4%). The isolated fungi were *Candida* spp. (3 strains) and *Acremonium* spp. All the detected viruses were herpes simplex virus type 1. When the etiology was studied according to the risk factors, coagulase-negative *Staphylococcus* (CoNS) strains were the most frequent microorganisms isolated except in patients with a history of surgical treatment among whom *Streptococcus viridans* was the most common microbe. Antibiotic susceptibility results are presented in Table 2. We found that 8.7% of the *Staphylococcus aureus* isolates and 26.4% of the CoNS isolates were methicillin-resistant. No significant trends were observed for methicillin-resistant strains ($p = 1.0$ for *S. aureus* and $p = 0.11$ for CoNS).

Table 1. Microorganisms isolated from infectious keratitis

| Microorganism | *n* | % |
|---|---|---|
| Bacteria | | |
| Gram-positive | 210 | 82.4 |
| CNS | 73 | 28.6 |
| *Propionibacterium* spp. | 50 | 19.6 |
| *Corynebacterium* spp. | 25 | 9.8 |
| *Streptococcus* spp. | 24 | 9.4 |
| *S. viridans* | 14 | 5.5 |
| *S. pneumoniae* | 9 | 3.5 |
| *S. agalactiae* | 1 | 0.4 |
| *Staphylococcus aureus* | 24 | 9.4 |
| *Bacillus* spp. | 8 | 3.1 |
| Others[1] | 6 | 2.4 |
| Gram-negative | 31 | 12.2 |
| *Pseudomonas aeruginosa* | 14 | 5.5 |
| *Enterobacteriaceae* | 10 | 3.9 |
| *Haemophilus influenzae* | 2 | 0.8 |
| *Neisseria* spp. | 2 | 0.8 |
| Others[2] | 3 | 1.2 |
| Total of bacteria | 241 | 94.5 |
| Fungi | | |
| *Candida* spp. | 3 | 1.2 |
| *Acremonium* spp. | 1 | 0.4 |
| Total of fungi | 4 | 1.6 |
| Virus | | |
| HSV type 1 | 10 | 3.9 |
| Total of microorganisms | 255 | 100 |

[1]: Including: *Enterococcus faecalis* (2), *Aerococcus urinae* (2), *Peptostreptococcus* spp. (1), and *Lactobacillus rhamnosus* (1).
[2]: Including: *Eikenella corrodens* (1), *Moraxella catarrhalis* (1), and *Pasteurella multocida* (1).
CNS, coagulase-negative *Staphylococcus*; HSV, herpes simplex virus.

The management of severe infectious keratitis requires a joint serious effort of ophthalmologists and microbiologists. In our study, 64.4% of corneal scrapes yielded positive results of cultures, showing superior diagnostic performance as compared with other series (5,6). When the sterile cultures were analyzed, 55.5% of the patients had a history of antibiotic treatment. It is possible that bacterial infections can be underdiagnosed. The frequent use of topical antimicrobial therapy in primary care before emergency ophthalmic referral as well as corneal scraping have been associated with lower percentages of positive cultures (7). The prevalence of polymicrobial keratitis was 10.4%. Previous studies have shown a frequency between 2.0% and 4.8% (8,9). A high index of suspicion of polymicrobial infection should be assigned to patients with multiple and systemic risk factors (8).

In accordance with other studies (5,7), gram-positive bacteria represented the most frequently isolated microorganisms (82.3%). The most common isolated pathogen was *S. epidermidis* (45 strains, prevalence 17.6%). The predominance of this organism has been reported by other groups (5,7). It is remarkable that the second and third most prevalent microorganisms were *Propionibacterium* spp. (19.6%) and *Corynebacterium* spp. (9.8%), respectively. These microorganisms may be considered contaminants because of their ubiquitous presence on the skin and normal conjunctiva. However, their pathogenic properties have been widely documented (10,11). We found that 8.7% of the *S. aureus* isolates and 26.4% of the CoNS isolates were methicillin-resistant; these rates are lower than those reported in similar studies (5). Another finding of interest is the small number of cases due to gram-negative microorganisms (12.1%), including *Pseudomonas aeruginosa* (5.4%). The predominance of gram-negative pathogens has been reported in other series and might be related to the rise of contact lens wear (6). We have not found this association. Although contact lens wear was the most frequent risk factor in our study, CoNS was the most common cause of infection in this group of patients.

Empiric broad-spectrum treatment with 2 fortified antibiotics (e.g., an aminoglycoside plus cephalosporin) or monotherapy with a fluoroquinolone is usually initiated before culture results are available (12). Some authors have suggested that fluoroquinolones are the first choice for empiric treatment (13). In addition, fluoroquinolones offer the theoretical advantage of good ocular penetration with a better tolerance profile (13). However, several studies have revealed increasing resistance to fourth-generation fluoroquinolones, and these drugs may provide insufficient protection against gram-positive pathogens (14). In our study, the prevalence of susceptibility to fluoroquinolones among gram-positive

Table 2. Antibiotic susceptibility of gram-positive and gram-negative bacteria

| Microorganism | PE (%) | CLO (%) | CFT (%) | CFZ (%) | CIP/LEV (%) | GM (%) | TO (%) | VAN (%) |
|---|---|---|---|---|---|---|---|---|
| Gram-positive bacteria | | | | | | | | |
| CNS[1] | | 73.6 | 73.6 | 73.6 | 76.7 | 84.9 | 87.3 | 100 |
| *Propionibacterium* spp. | 97.4 | | 100 | 100 | 86.5 | | | 100 |
| *Corynebacterium* spp. | 100 | | 100 | 100 | 91.7 | | | 100 |
| *Staphylococcus aureus*[2] | | 91.3 | 91.3 | 91.3 | 87.5 | 95.6 | 91.3 | 100 |
| *Streptococcus viridans* | 92.8 | | 100 | 100 | 92.3 | | | 100 |
| *Streptococcus pneumoniae* | 77.8 | | 100 | 100 | 100 | | | 100 |
| *Bacillus* spp. | 75 | | 66.7 | 66.7 | 100 | 100 | | 100 |
| Others[3] | 85.7 | | 80 | 80 | 83.3 | | | 85.7 |
| Total of gram-positive bacteria | 92.2 | 77.9 | 83.7 | 83.7 | 84.6 | 87.9 | 88.3 | 99.4 |
| Gram-negative bacteria | | | | | | | | |
| *Pseudomonas aeruginosa* | | | | 64.3 | 100 | 100 | 100 | |
| *Enterobacteriaceae* | | | 100 | 100 | 90 | 90 | 100 | |
| Others[4] | | | 100 | 100 | 85.7 | 100 | 100 | |
| Total of gram-negative bacteria | | | 55.3 | 83.3 | 93.5 | 96.3 | 100 | |

[1]: 19 strains were methicillin-resistant (26.4%).
[2]: 2 strains were methicillin-resistant (8.7%).
[3]: Including: *Streptococcus agalactiae* (1), *Peptostreptococcus* spp. (1), *Enterococcus faecalis* (2), *Aerococcus* spp. (2), and *Lactobacillus rhamnosus* (1).
[4]: Including: *Haemophilus influenzae* (2), *Neisseria* spp. (2), *Eikenella corrodens* (1), *Moraxella catarrhalis* (1), and *Pasteurella multocida* (1).
CNS, coagulase-negative *Staphylococcus*; PE, penicillin; CLO, cloxacillin; CFT, cefotaxime; CFZ, ceftazidime; CIP, ciprofloxacin; LEV, levofloxacin; GM, gentamicin; TO, tobramycin; VAN, vancomycin.

microorganisms was 84.4%. Vancomycin (VCM), gentamicin (GM), and tobramycin (TO) showed higher percentages (99.4%, 87.9%, and 88.3%, respectively). Our results indicate that VCM is the best option for treating infections caused by gram-positive microorganisms. Gentamicin and TO were highly effective against gram-negative microorganisms. Moreover, all gram-negative isolates, including *P. aeruginosa*, were susceptible to TO. Based on our findings, it seems reasonable to consider a combination of VCM and TO as the initial empiric treatment in cases of bacterial keratitis originating in our area. This combination should be seriously considered for severe bacterial keratitis and pretreated cases in the community that have not responded to treatment. However, VCM and aminoglycosides are known to have significant ocular-surface toxicity and low tolerability (15). Recently, some authors advocated topical linezolid as an alternative to VCM because the former has lower toxicity and covers gram-positive microorganisms (16).

In conclusion, our study revealed strong predominance of gram-positive microorganisms among patients with bacterial keratitis. We suggest empirically treating bacterial keratitis originating in our area with VCM and TO, especially severe bacterial keratitis and pretreated cases in the community without a clinical response.

**Conflict of interest** None to declare.

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