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Infectious keratitis in South Australia: Emerging resistance to cephazolin

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PURPOSE. *To analyze the microbiologic spectrum and patterns of resistance of infectious keratitis in patients treated at a tertiary hospital in South Australia.*
METHODS. *Retrospective review of microbiology laboratory records of all patients with infectious keratitis who had corneal scrapings, from 1998 to 2003. All records were subsequently reviewed for Gram staining and culture results, as well as antibiotic sensitivity and resistance.*
RESULTS. *Positive corneal cultures were obtained in 134 out of 211 patients who had corneal scrapings (63.5%). Coagulase negative* Staphylococcus *was the commonest pathogen identified (29.8% of positive cultures), followed by* Staphylococcus aureus *(18.7%),* Pseudomonas aeruginosa *(12.7%),* Moraxella *(6.7%),* Streptococcus pneumonia *(6.0%), and fungal keratitis (5.2%). In 43.3% of culture positive cases, the organisms were also identified in Gram stain, and in all these cases there was a full correlation between the two methods. In vitro sensitivities were highest for gentamicin. Fourteen cases (35%) of coagulase negative* Staphylococcus *were found to be resistant to cephazolin. No ciprofloxacin resistance was identified in all* Pseudomonas *isolates tested.*
CONCLUSIONS. Staphylococcus *species continue to be the commonest causative organism for infectious keratitis; however, there is an emerging resistance to cephazolin, which is commonly used as the first-line antibiotic for Gram-positive cocci. (Eur J Ophthalmol 2005; 15: 23-6)*

KEY WORDS. *Keratitis, Bacterial, Infection, Antibiotics, Resistance*

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INTRODUCTION

Infectious keratitis is an ophthalmologic emergency that may be associated with significant ocular morbidity and result in sight-threatening consequences. Early clinical and laboratory recognition of the responsible pathogens may enable effective antibiotic treatment and reduce ocular morbidity resulting in long term corneal scarring and visual loss (1, 2).

Corneal scrapings and cultures are the most important tools in deciding the appropriate antibiotic therapy. However, it was shown recently in several reports that the spectrum of pathogens that may cause infectious keratitis may vary not only because of host factors but also according to geographic location (3-10). This variability in etiologic organisms according to geographic location may also be associated with different patterns of antibiotic resistance, a fact that may be important when treatment is considered. This is a 5-year study of all cases of infectious keratitis treated at a tertiary hospital in South Australia. All patients included had corneal scrapings done prior to commencing antibiotic treatment, and this study analyzes the microbiologic spectrum and patterns of resistance in these cases of keratitis.

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METHODS

Microbiology laboratory records and patients' notes were retrospectively reviewed for all cases of infectious keratitis (bacterial or fungal) that underwent corneal scrapings, from December 1998 to December 2003, at the Royal Adelaide Hospital, a tertiary hospital in South Australia. All corneal scrapings were taken at the ophthalmology clinic using a Kimura spatula or needle. The material collected was streaked over agar plates (blood and chocolate). Sabouraud's agar plates were used to enhance fungal growth, selective media for aerobic and anaerobic bacteria, as well as non-nutrient agar with *Escherichia coli* and Lowenstein-Jensen slant in cases of suspected acanthamoeba or mycobacterium, respectively. Gram-stained smears were done in all cases. All agar plates, selective media, and smears were immediately transferred to the microbiology laboratory at the Royal Adelaide Hospital. Positive cultures were tested for various antibiotic drugs, including penicillins, aminoglycosides (tobramycin, neomycin, amikacin, and gentamicin), cephalosporins (cephazolin), macrolides (erythromycin), tetracycline, chloramphenicol, and fluoroquinolones (ciprofloxacin). All patients' case notes were reviewed for demographics and clinical data. In addition, culture and Gram stain correlations and reliability were compared in all cases.

TABLE I - CULTURE ISOLATED INFECTIOUS ORGANISMS IN PATIENTS WITH INFECTIOUS KERATITIS

Number of positive cultures (%)	Organism
Gram - positive cocci	
40 (29.8)	Coagulase-negative *Staphylococcus*
25 (18.7)	*Staphylococcus aureus*
8 (6.0)	*Streptococcus pneumonia*
4 (3.0)	*Streptococcus viridans*
Gram - positive rods	
8 (6.0)	*Corynebacterium macginley*
2 (1.5)	*Bacillus*
2 (1.5)	*Propionibacterium acnes*
Gram - negative cocci	
9 (6.7)	*Moraxella*
1 (0.7)	*Neisseria*
Gram - negative rods	
17 (12.7)	*Pseudomonas aeruginosa*
2 (1.5)	*Proteus mirabilis*
3 (2.2)	*Serratia*
2 (1.5)	*Enterobacteriaceae*
2 (1.5)	*Acinetobacter*
2 (1.5)	*Diphtheroids*
Actinomycetales	
3 (2.2)	*Aspergillus fumigatus*
2 (1.5)	*Scedosporium apiospermum*
2 (1.5)	*Candida* species

RESULTS

There were 211 patients (127 males and 84 females), mean age: 59.6 years (range: 16 to 95 years), who had corneal scrapings for infectious keratitis over the 5-year period. There were two main categories of patients with corneal ulcers seen at our institution: the first consisted of patients seen for the first time in the emergency department, and the second of patients referred to the eye clinic from community ophthalmologists and general practitioners.

Topical antibiotics were stopped for 6 hours prior to scrapings in all patients who were already on topical antibiotic therapy.

A total of 134 cases (63.5%) had positive cultures. *Staphylococcus* coagulase negative (29.8% of positive cultures) was the commonest pathogen identified. The other common organisms, in order of decreasing frequency, were *Staphylococcus aureus* (18.7% of positive cultures), *Pseudomonas aeruginosa* (12.7%), *Moraxella* (6.7%), and *Streptococcus pneumonia* (6.0%) (Tab. I).

In 19 cases there was more than one organism identified on culture. The commonest organism in these mixed infections was coagulase-negative *Staphylococcus*. Fungal keratitis (*Aspergillus fumigatus, Scedosporium apiospermum*, and *Candida* species) accounted for 5.2% of cases. There was no change in pathogen frequency over the 5 years of the study.

The results of Gram staining were compared with culture results. In 58 cases (43.3%) of culture positive results, the organisms were also identified using Gram stain.

In all these cases there was a full correlation between the culture and Gram staining. In vitro sensitivities to commonly used topical antibiotics showed highest sensitivity for gentamicin and a high frequency of resistance to cephazolin. Fourteen cases (35%) of coagulase-negative *Staphylococcus* infection were found to be resistant to latter antibiotic. No resistance to fluoroquinolones was identified in any *Pseudomonas* isolates tested.

DISCUSSION

Infectious keratitis is one of the most visually threatening ocular infectious pathologies. The avascular corneal stroma is particularly susceptible to bacterial infection, and many patients have a poor clinical outcome if aggressive and appropriate therapy is not promptly initiated. Effective treatment depends on early diagnosis, initial intensive treatment with broad-spectrum antibiotics, followed by a more specific treatment after recognition of the responsible pathogen diagnosed by smears and cultures from corneal scrapings (1, 2).

The spectrum of pathogens and resistance patterns varies between different geographic locations in addition to shifting trends with time (Tab. II). In a report by Alexandrakis et al (4) evaluating cases of bacterial keratitis over a period of 9 years (1990 to 1998) in South Florida, they found an increase in the incidence of *S. aureus* isolates over the years (although *Pseudomonas* was the most common, but with a decreasing incidence) and decreased effectiveness of fluoroquinolones. Varaprasathan et al (5) compared the relative frequencies of corneal pathogens in northern California between 1976 and 1999 to the results of a previous study done in the same institution from 1948 to 1976. They found a decrease in isolating *Streptococcus pneumonia* and *Pseudomonas*, and an increasing frequency of *Serratia marcescens*. Fong et al (6) analyzed the spectrum of microbial keratitis in Taiwan between 1992 and 2001. *Pseudomonas* species were the most common isolates (37.7%), followed by fungi and staphylococci (8.4% only). Laspina et al (7) evaluated the epidemiologic characteristics of infectious keratitis in Paraguay over a period of 13 years (1988-2001). They found that coagulase negative *Staphylococcus* was the most common pathogen isolated (25%) followed by *S. aureus* (23%) and *Pseudomonas* (13%). Chalita et al (8) assessed in vitro susceptibility of ocular isolates in patients in Brazil between 1985 and 2000. They found a decrease in in vitro susceptibility to gentamicin, tobramycin, and cephalothin. Fluoroquinolones and chloramphenicol maintained good in vitro susceptibility to all pathogens tested. These different reports emphasize the importance of recognizing the specific pattern of common pathogens in every geographic area. Knowing this pattern may help in choosing the initial empiric therapy prior to final laboratory results or in cases where no pathogen was identified.

Our study was performed in South Australia, in an area with a temperate climate. The distribution of organisms detected is similar to some other studies in which *S. aureus* and coagulase-negative *Staphylococcus* were found to be the most frequent organisms (3, 7, 9, 10). No ciprofloxacin resistance was detected in our series, indicating that fluoroquinolones monotherapy is an effective treatment for

TABLE II - SUMMARY OF STUDIES EVALUATING COMMON PATHOGENS IN BACTERIAL KERATITIS

Characteristics	Alexandrakis et al (4)	Varaprasathan et al (5)	Fong et al (6)	Laspina et al (7)	Chalita et al (8)	Current study
Study period	1990-1998	1976-1999	1992-2001	1988-2001	1985-2000	1998-2003
Study place	South Florida	Northern California	Taiwan	Paraguay	Brazil	South Australia
Common isolates	*Pseudomonas* (25.7%) *Staphylococcus aureus* (19.4%)	*Staphylococcus aureus* (20%)	*Pseudomonas* (37.7%)	Coagulase -negative *Staphylococcus* (25%) *Staphylococcus aureus* (23%)	N-A	Coagulase -negative *Staphylococcus* (29.8%) *Staphylococcus aureus* (18.7%)
Antibiotic resistance	Increased resistance to fluoroquinolones	N-A	N-A	N-A	Increased resistance to cephalothin	Increased resistance to cephazolin

Staph= *Staphylococcus*; Coag. neg.= Coagulase negative; N-A= Data not available corneal ulcer in our region. However, increasing reports of bacterial resistance to fluoroquinolones (4, 11) emphasize the importance of careful monitoring of patients treated with fluoroquinolones monotherapy as an empiric choice. In addition, it is mandatory to perform periodic testing of bacteria isolated from patients with bacterial keratitis against available fluoroquinolones in order to establish the current resistance pattern (11).

In 43.3% of culture-positive cases in our series the organisms were also identified by Gram staining, and there was a full correlation between these two methods. These findings are consistent with other studies, supporting the observation that Gram staining should always be combined with culture when trying to isolate the specific pathogen and that Gram staining alone is an insufficient indicator to a sterile or nonbacterial ulcer (3, 6, 12).

We found a 35% resistance rate to cephazolin by coagulase-negative *Staphylococcus*. This first-generation cephalosporin is commonly used as the first-line antibiotic for Gram-positive cocci and as an empiric therapy in cases with no specific pathogen isolated. Chalita et al (8) also reported a decrease of *S. aureus* and *Pseudomonas* susceptibility to another first generation cephalosporin (cephalothin) over a period of 15 years. This decrease may be attributed to the frequent use of this drug in the treatment of bacterial keratitis. However, we must consider that cases seen in our hospital, a tertiary referral center, are possibly caused by more virulent pathogens, which are more resistant to the common antibiotic treatment regimens given by general ophthalmologists and general practitioners.

Periodic susceptibility surveys such as our study are important to detect and document changes in resistance patterns and provide clinically useful information. While reviewing the literature, we found no previous studies evaluating the spectrum of pathogens and antibiotic resistance of infectious keratitis in South Australia. However, our study has limitations, insofar as it is a retrospective study with possible selection biases created by the referral of patients with more resistant pathogens. This population of hospital patients may not represent the true community spectrum of pathogens and resistance.

In conclusion, emerging resistance to first generation cephalosporins, in the treatment of infectious keratitis, warrants careful monitoring of patients treated empirically with these drugs. Periodic antibiotic testing will enable early detection of any change in resistance pattern and help direct appropriate initial therapy.

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