A study of bacterial isolates from corneal specimens and their antibiotic resistance profile

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ABSTRACT

Objective: We aim to examine the spectrum of bacteria causing corneal infections and their antibiotic susceptibility patterns. This will serve as a guideline for empiric therapy of corneal infections.

Methods: We conducted the study over a period of 18 months from March 2001 through December 2002 in King Abdul-Aziz University Hospital, Riyadh, Kingdom of Saudi Arabia. Corneal specimens taken from 200 patients were inoculated directly onto different types of media. The isolates were identified and then tested against the appropriate topical or systemic antibiotics.

Result: Sixty-seven (33.5%) of the total specimens were culture positive and 133 (66.5%) were culture negative. Fourteen (7%) of these showed organisms in the Gram stained smears and correlated well with the culture reports. Of the 67 positive cultures, 53 (79.1%) were Gram-positive bacteria mostly coagulase-negative *Staphylococci* 29 (43.3%) followed by *Streptococcus pneumoniae* (*S. pneumoniae*) 13 (19.4%). Among Gram-negative bacteria 14 (20.9%), *Pseudomonas aeruginosa (P. aeruginosa)* 10 (14.9%) was the predominant isolate. All the isolates were sensitive to ofloxacin and the commonly used ocular antibiotics.

Conclusion: All the isolated bacteria were sensitive to ofloxacin, a fluoroquinolone. Having marked potency for broad-spectrum activity against both Gram-positive and Gram-negative bacteria, make the fluoroquinolones especially the newer generations, a potential single drug therapy for corneal infections.

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Corneal infection is a serious condition with significant morbidity that requires prompt diagnosis and therapy. Microbial keratitis has become a major public health problem and should therefore be approached thoughtfully. Causative agents include bacteria, fungi, viruses and protozoa. Since the clinical outcome could be critical, there is a need for initiation of prompt and aggressive therapy. In spite of this approach, many cases of microbial keratitis progressed to blindness. Some of the major predisposing factors causing corneal infections are: contact lens wear, corneal trauma, viral keratitis, dry eye syndrome, surgery (including laser), eyelid abnormalities (entropion or ectropion), bullous keratopathy, corneal transplantation and concomitant systemic diseases such as diabetes mellitus and rheumatoid arthritis.[1,2]

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Most microbes cannot penetrate the intact epithelial layer of the cornea protected by the eyelids and tear. The eyelids provide mechanical protection of the corneal surface and the tear film contains several immunologically active substances such as lysozyme, lactoferrin, beta-lysin, ceruloplasmin, complement and immunoglobulins (IgA, IgG and IgM).

Ocular infection would only occur when one of the mechanisms had failed to function or trauma in some form had breached the mechanical defense.[3]

The aim of this study was to examine the various bacteria causing corneal infections and to illustrate their antibiotic patterns. Furthermore, we aim to conclude a guideline for empiric corneal therapy.

Methods. This retrospective study was undertaken on bacterial isolates from corneal specimens of 200 patients over a period of 18 months from March 2001 to December 2002. All the information related to this study was reviewed from the microbiological laboratory computer database. Patients with viral, protozoal or fungal infections were excluded. The study involved 144 male and 56 female. The age distribution of these patients range between 2-87 years. Most of the patients age ranged from 21-50 years (116 patients).

The specimens consisted of 102 corneal scrapings from corneal ulcers and 98 swabs from suspected corneal infections. Each specimen was collected and inoculated directly on the following media: 2 sheep blood agar plates, chocolate agar plate, brain heart infusion broth and Sabouraud dextrose agar slope and the tips of surgical spears and swabs with transport medium (Transwab for aerobes and anaerobes, Medical Wire and Equipment Co. Ltd., UK) were broken into thioglycollate broth U.S.P. Alternative (Oxoid Ltd, Basingstoke, UK).

Culture media was accompanied by 2 smears for Gram and Giemsa stains for each specimen and examined for bacteria. One sheep blood agar and chocolate agar plates were incubated at 35˚C in 5% carbon dioxide for 3 days, the second sheep blood agar was maintained at 35˚C anaerobically for 3 days in a mixture of 5% carbon-dioxide, 10% hydrogen and 85% nitrogen. The thioglycollate broth was maintained at 35˚C for 2 weeks, the brain heart infusion broth slope at 35˚C and Sabouraud dextrose agar slope 30˚C for 4 weeks. All the media were obtained from Oxoid Ltd., Basingstoke, United Kingdom and prepared according to the manufacturer's instructions.

The isolated bacteria were identified by standard procedures. Gram-negative bacilli was identified by API 20 E (API 20 NE if non-fermentative Gram-negative bacilli), *Streptococcus* species (other than *S. pneumoniae*) by API 20 Strep and any anaerobes by API 20 A kits (bioMérieux, Sa, France). The *S. pneumoniae* was identified by Optochin 5 μg sensitivity disc (Oxoid Ltd. Basingstoke, UK).

The bacterial isolates were tested for their resistance against the following commonly used ocular antibiotics: penicillin G, erythromycin, ofloxacin, vancomycin, ceftriaxone, chloramphenicol, cefazolin, fusidic acid and bacitracin. For Gram-positive bacteria; ampicillin, cefazolin, ceftazidime, ceftriaxone, ofloxacin, gentamicin, polymyxin B was used on the isolates and chloramphenicol for Gram-negative bacteria, using standard disc diffusion method (Stokes method).[4] The susceptibility testing was performed according to the National Committee for Clinical Laboratory Standards.[5] Antibiotics discs were obtained from Mast Diagnostics (Bootle, Merseyside, UK). The cut off criteria for systemic infections was used to interpret the sensitivity results.

Table 1 - Correlation between predisposing factors and organisms isolated.

Bacteria isolated n=67	Predisposing factors							
	Contact lens	Corneal trauma	Diabetes mellitus	Viral keratitis	Hypo-thyroidism	Corneal transplant	Corneal burns	Other corneal disorders
CNS	6	14	-	-	-	1	1	7
S. pneumoniae	-	6	4	-	1	-	-	2
Staph. aureus	-	4	2	-	-	-	-	3
V. streptococci	-	2	-	-	-	-	-	-
P. aeruginosa	7	1	2	-	-	-	-	-
M. lacunata	-	-	1	-	-	-	-	1
H. influenzae	-	1	-	-	-	-	-	-
N. spp	-	1	-	-	-	-	-	-

CNS - Coagulase-negative *staphylococci*, *S. pneumoniae* - *Streptococcus pneumoniae*, *Staph. aureus* - *Staphylococcus aureus*, *V. streptococci* - *Viridans streptococci*, *P. aeruginosa* - *Pseudomonas aeruginosa*, *M. lacunata* - *Moraxella lacunata*, *H. influenzae* - *Haemophilus influenzae*, *N.* spp - *Neisseria* species

Table 2 - Frequency of predisposing factors in this study causing corneal lesions N=200

Predisposing factors	n	(%)
Contact lens wear	25	(12.5)
Corneal trauma	114	(57)
Diabetes mellitus	20	(10)
Viral keratitis	4	(2)
Hypothyroidism	2	(1)
Corneal transplant	2	(1)
Chemical burns	2	(1)
Other corneal disorders, such as dry eye syndrome, corneal dystrophy	31	(15.5)

Table 3 - Spectrum of 67 organisms isolated from positive cultures.

Organisms	n	(%)
Gram positive organisms	53	(79.1)
CNS	29	(43.3)
S. pneumoniae	13	(19.4)
Staph. aureus	9	(13.4
V. streptococci	2	(3)
Gram negative organisms	14	(20.9)
P. aeruginosa	10	(14.9)
M. lacunata	2	(3)
H. influenzae	1	(1.5)
N. spp	1	(1.5)

CNS - Coagulase-negative *staphylococci, S. pneumoniae - Streptococcus pneumoniae, Staph. aureus - Staphylococcus aureus, V. streptococci -Viridans streptococci, P. aeruginosa - Pseudomonas aeruginosa, M. lacunata - Moraxella lacunata, H. influenzae - Haemophilus influenzae, N. spp - Neisseria*

Table 4 - Percentage of antibiotic resistance profile of isolates.

Antimicrobial agents	PG	E	OFL	CZ	CRO	VA	CHL	NE	BAC
Gram positive bacteria									
S. pneumoniae (n=13)	38.5	7.7	0	38.5	0	0	0	100	0
Staph. aureus (n=9)	100	0	0	33.3	NT	0	0	0	0
CNS (n=29)	89.7	37.9	0	72.4	NT	0	3.4	17.2	0
V. streptococci (n=2)	50	50	0	0	0	0	0	100	0
Antimicrobial agents	**CZ**	**CRO**	**CAZ**	**OFL**	**GM**	**NE**	**PB**	**CHL**	**AMP**
Gram negative bacteria									
P. aeruginosa (n=10)	100	NT	0	0	0	100	0	100	NT
M. lacunata (n=2)	100	0	NT	0	0	0	0	0	100
H. influenzae (n=1)	100	0	NT	0	0	0	0	0	0
N. spp (n=1)	100	0	NT	0	0	0	0	0	100

PG - Penicillin G, AMP - Ampicillin, E - Erythromycin, OFL - Ofloxacin, CZ - Cefazolin, CRO - Ceftriaxone, CAZ - Ceftazidime, VA - Vancomycin, GM - Gentamicin, NE - Neomycin, PB - Polymyxin B, CHL - Chloramphenicol, BAC - Bacitracin, NT - Not tested *S.pneumoniae – Streptococcus pneumoniae, Staph. aureus-Staphylococcus aureus,* CNS-Coagulase-negative *staphylococci, V. streptococci.- Viridans streptococci, P. aeruginosa-Pseudomonas aeruginosa, M. lacunata-Moraxella lacunata, H. influenzae- Haemophilus influenzae, N.* spp – *Neisseria* species

Result. Results of 200 corneal specimens were reviewed from the laboratory records involving 144 male (72%) and 56 female (28%). One hundred and two (51%) from the right eye and 98 (49%) from left eye including 25 (12.5%) from contact lens wearers. The correlation between predisposing factors and organism isolated were illustrated in **Table 1**. Frequencies of predisposing factors were identified and are summarized in **Table 2**. Out of the 200 corneal specimens reviewed, a total of 67 specimens (33.5%) were culture positive growing identifiable organisms and 133 specimens (66.5%) yielded no growth. The spectrums of isolated bacteria are shown in **Table 3**. Out of the 67 positive cultures, 53 (79.1%) yielded Gram-positive organisms mostly coagulase-negative *Staphylococci* (all were *Staphylococcus epidermidis*) followed by Gram-negative bacilli 14 (20.9%) mostly *P. aeruginosa*.

Organisms were found on examination with Gram stain in 14 (7%) of the 200 specimens (20.9% of the positive cultures).

All the specimens with organisms found in the Gram stain correlated well with the culture reports. The organisms frequently isolated were coagulase-negative *Staphylococci* followed by *S. pneumoniae, P. aeruginosa, Staphylococcus aureus (Staph. aureus).* In this study all *Staph. aureus* isolated were sensitive to fusidic acid. All the coagulase-negative *Staphylococci* except one were recovered from thioglycollate broth. Thirteen (52%) of the 25 corneal specimens from contact lens wearers were culture positive, yielding *P. aeruginosa* 7 (53.8%) as the predominant organism. Most of the isolates were sensitive to the commonly

used ocular antibiotics especially ofloxacin. **Table 4** shows percentage of antibiotic resistance profile of the isolates.

Discussion. The management of corneal infections includes the identification of causative agents by Gram and Giemsa stains, cultural methods, immediate treatment with broad spectrum antibiotics, readiness by the ophthalmologists to modify treatment based on the susceptibility pattern of the causative agents. This would also demands selection of proper concentration of antibiotic and mode of administration, and taking bacterial resistance and drug toxicity into consideration.

Our rate of recovery of organisms from corneal specimens was comparatively low. The reason for this is that patients could have been given antimicrobial therapy in other hospitals before being referred to this hospital or most patients come to the emergency department immediately after trauma giving less time for the bacteria to multiply.

Organisms were found in the Gram stain of only 14 (7%) of the total number of specimens and 20.9% of the positive cultures. Forty percent of *P. aeruginosa* isolated and 31% of *S. pneumoniae* isolated had corresponding bacteria in the Gram stained smears and correlated well with the culture reports. This showed that Gram stain is in effect specific but not sensitive. The low sensitivity of Gram stain observed in this study supports the conclusion reached by Cheung and Slomovic[2] that Gram stain may not be useful for the management of corneal infections. This study showed that *P. aeruginosa* is the predominant organism isolated from contact lens wearer which support the work carried out by Tabbara et al[6] and others.[7-9]

In this study, coagulase-negative *Staphylococci* were the most common organism isolated (29 [43.3%]) supporting other studies, that although coagulase-negative *Staphylococci* are indigenous organisms, they are becoming the most common isolates in corneal infections as shown in other studies.[2,10-12]

All the coagulase-negative *Staphylococci* except one were recovered from thioglycollate broth. Thioglycolate medium was previously reported by Rombaux et al[13] that this medium support the growth of both aerobic and anaerobic organisms including coagulase negative *Staphylococcus*.

A probable reason why the coagulase-negative *Staphylococci* were only recovered from the thioglycollate broth and not from the culture plates could be that the tips of the surgical spears and swabs were cut directly into the thioglycollate broth or antibiotics already given to the patients might have been neutralized or diluted out by the thioglycollate broth thereby increasing the chances of isolation or could be the number of the organisms present in the specimen.

All the isolates in this in vitro study, both Gram-positive and Gram-negative organisms were sensitive to ofloxacin, a fluoroquinolone and all the Gram-positive organisms were sensitive to vancomycin.

Recently, fluoroquinolones are becoming increasingly popular for the ophthalmologists as single drug therapy in corneal infections since they appear to be effective against both Gram-positive and Gram-negative organisms including *P. aeruginosa* and Methicillin Resistant *Staph. aureus* (MRSA) and therefore be used as single drug therapy.[14-16] They appear to compare favorable with the combination of fortified cefazolin and aminoglycoside for the treatment of corneal infections.[14,15] In vitro, none of the isolates was resistant to ofloxacin, nevertheless, recent report had shown emerging fluoroquinolones resistance in both Gram positive and gram negative bacteria especially *Staph. aureus,* MRSA, *P. aeruginosa* and reduced sensitivity to S. pneumoniae.[17] However, we still see a major role of fluoroquinolones in the management of corneal infections despite the emergence of resistance. The newer generations of fluoroquinolones for example, levofloxacin, gatifloxacin and moxifloxacin with their more expanded spectrum of antibacterial activity against Gram positive and Gram negative bacteria including non-tuberculous mycobacteria and improved pharmacokinetic properties would probably replace fortified cefazolin and aminoglycoside as a topical single drug therapy. In severe corneal infections and trauma we suggest that the initial empiric treatment could be combination of topically administered fluoroquinolone and vancomycin. In deep penetrating trauma vancomycin and ceftazidime should also be given systemically according to the observation from this study. Vancomycin could be used for highly resistance Gram positive cocci such as, *Staph. aureus* and *Streptococcus fecalis*.[18,19]

Ophthalmologists should be aware of the potential gaps in the spectrum of antibacterial activity especially among *Enterococci, Pseudomonas* species, penicillin-resistant *S. pneumoniae* and other organisms to fluoroquinolones and should be willing to modify the treatment after receiving culture and sensitivity result of the isolates from the corneal specimens. In this study, all isolates of *P. aeruginosa* were sensitive to ceftazidime, gentamicin and ofloxacin. This study revealed the following interesting observations, which could be of epidemiological significance: 1. That

corneal infections occur mostly in patients between the ages of 21-50 years age group as this may be the age of immense activity of various sorts. 2. Most of the *P. aeruginosa* were isolated from females distributed throughout all age groups. Future studies are needed to verify these observations.

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