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Clinico-etiological profile and antimicrobial susceptibility pattern of bacterial keratitis in the era of automated platforms

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Background: Microbial keratitis is one of the most significant causes of blindness in our country. Knowledge regarding the aetiology and antibiotic sensitivity pattern in a specific region is crucial for the ideal management of these infections. **Materials and Methods:** A prospective study where corneal scrapings from 161 suspected cases of microbial keratitis were collected and were subjected to direct microscopy by gram stain and bacterial culture and identification as well as antibiotic sensitivity testing. **Results:** A total of 57 samples turned out to be positive out of the total 161 and were shared equally by both gram-positive and gram-negative bacteria. All these culture-positive bacteria were also identified by MALDI TOF-MS and were speciated. Few rare organisms which could not be identified by conventional means were also recovered using the same. Most of the gram-positive isolates showed good sensitivity to vancomycin and ciprofloxacin whereas Pseudomonas spp was found to be resistant to the aminoglycosides**. Conclusion:** Right knowledge about the local profile of bacterial causes of keratitis along with its antibiotic resistance pattern will help the clinicians immensely and help them to initiate the correct empirical therapy bases on the smear results without wasting crucial time.

Keywords: Microbial keratitis, Antibiotic sensitivity, Gram-positive

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Introduction

Keratitis is the term used for inflammations of the cornea. [1] In developing countries, these corneal infections are the second most important cause of ocular blindness after unoperated cataract. [2] Microbial keratitis is an ophthalmic emergency that requires urgent attention and can be caused by bacteria, fungi, viruses or parasites. Bacterial keratitis is rarely seen in the absence of predisposing factors [3].

Until recently, most cases of bacterial keratitis were associated with ocular trauma and diseases of the ocular surface. However, the widespread use of contact lens has increased the incidence of bacterial keratitis tremendously. These bacteria multiply in the contact lens cases where they are protected from disinfection by bacterial biofilm.

Smear and culture of the corneal scrapings remain the gold standard for identification of the offending organism. [4] With the advent of Matrix-Assisted Laser Desorption/Ionization-Time Of Flight (MALDI-TOF) the turnaround time for identification of the bacterial agents has reduced to half and also become more accurate and detailed (species level). It enables us to identify the rarest of organisms that are difficult to be picked up by conventional identification methods.

Initial therapy should be initiated based on the smear results and targeted antimicrobial therapy later on can be backed up by the culture results. With the rampant use of broad-spectrum antibiotics, wide variation in the microbial spectrum and antibiotic susceptibility pattern has been observed. [5] The prevalence of different bacterial agents has also been influenced by geographic and climatic factors. Many differences in keratitis profile such as type of organism isolated, susceptibility and resistance pattern have been noted between rural and urban populations, in western and in developing countries [6].

Therefore, knowledge of the local antibiotic sensitivity pattern is the need of the hour to provide the right empirical management of bacterial keratitis. Because of the meagre data available from this part of the country, this study aims to determine the etiology and most importantly the antibiotic sensitivity pattern of bacterial keratitis from the highest tertiary care centre catering to the state of Uttarakhand and also the adjoining states.

Materials and methods

This prospective cross-sectional study was carried out in a tertiary healthcare teaching institute from February 2019 to January 2020. Ethical clearance was taken from the Institute Ethics Committee. The patients were clinically examined by experienced clinicians and after taking informed consent, corneal scraping samples were collected from 161 clinically suspected cases of Microbial keratitis (MK). Inclusion criteria were the presence of signs of MK in slit-lamp examination (i.e., epithelial defect, underlying stromal infiltrate with signs of acute inflammation e.g., circumcorneal congestion, stromal infiltrates, hypopyon, scarring or perforation).

Corneal scrapings were obtained under topical anaesthesia by scraping the base and edges of the ulcer under the magnification of a slit lamp, using a sterile Bard-Parker blade. The patient's age, gender, occupation, history of any predisposing factor like trauma, systemic illness, past and current use of topical medicines or use of contact lens use were entered in the Microsoft Excel sheets. Samples were sent to the ocular Microbiology section and were processed by standard procedures for the diagnosis of causative pathogens.

Microbiological processing of the corneal scrapings included smear preparation and inoculation onto culture media. The scrapping material was inoculated onto blood agar, Chocolate agar and smeared onto a slide for Gram stain. The material was also inoculated in glucose broth. All media were incubated aerobically at 37^{0}C except Chocolate agar (incubated in 5% CO_2 at 37^{0}C). The media were examined daily for 7 days.

The cultures were considered positive if the growth of the same organism was demonstrated in more than one solid media, or growth on one medium was consistent with direct microscopy findings, or confluent growth was obtained on inoculated single solid medium, or the same organism was grown from repeated corneal scrapings. The bacteria isolated had been identified by standard biochemical test methods and MALDI TOF-MS (Bruker Biotyper Microflex, MA, USA).

Cultured bacterial isolates were subjected to antimicrobial testing to a range of antibiotics commonly used in the treatment of corneal ulcer. Antibiotic sensitivity was performed by Kirby Bauer's disk diffusion method as per the Clinical and

Laboratory Standards Institute (CLSI) guidelines, which classify organisms as susceptible, resistant, or intermediate susceptible to antibiotics. For data analysis in this study, organisms with intermediate susceptibility were grouped as susceptible.

Results

This study was conducted jointly in the Department of Microbiology and Ophthalmology in a tertiary care centre located in the state of Uttarakhand. A total of 161 samples from clinically suspected keratitis cases were collected. On Gram staining, Gram-positive cocci in 17% (n=28/161) of cases and Gram-negative bacilli in 17% (n=28/161) of cases were seen. Based on culture growth obtained, infective etiology could be established in 35 % of cases (n=57/161) of cases according to the predefined criteria.

Table: 1. summarizes identified causative bacterial agents responsible for the infection. Of all the bacterial isolates obtained, the most common isolate was *Coagulase-negative Staphylococcus* Species (20/57*),* followed by *Pseudomonas aeruginosa* (13/57), *Acinetobacter baumannii complex* (9/57), *Streptococcus pneumonie* (4/57), *Staphylococcus aureus* (4/57), *Escherichia coli* (3/57), *Klebsiella pneumonia* (2/57), *Yersinia pseudotuberculosis* (1/57) *and Nocardia* (1/57). Confirmatory Identification was also done by MALDI TOF-MS (Bruker Biotyper Microflex, MA, USA) in all the 57 isolates.

Table 2. enumerates the percentage of strains susceptible to different anti-bacterial ophthalmic agents. To summarize, 47.1% of isolates were sensitive to Chloramphenicol, 53.6 % to Erythromycin, 55.7% to Gentamicin, 59.6% to Tobramycin, 62.5% to Cotrimoxazole, 65.4% to Amikacin, 68.4% to Doxycycline, 75% of the isolates to Azithromycin, 84.6% to ciprofloxacin, 85.3% to Tetracycline, 89.3 % to Levofloxacin, and 100% of the isolates were sensitive to vancomycin and polymyxins. Antimicrobial susceptibility was not put for *Nocardia,* as the testing method was not validated for the same.

Table-1: Distribution of identified pathogens from infective keratitis cases in 1 year study period (n=57)

PATHOGENS	IDENTIFICATION BY CONVENTIONAL METHOD	IDENTIFIED BY MALDI TOF -MS (≥1.5 confidence interval)
Bacteria		
Coagulase-negative Staphylococcus species	20	Staphylococcus epidermidis (13) Staphylococcus haemolyticus (6) Staphylococcus hominis (1)
Pseudomonas aeruginosa	13	Pseudomonas aeruginosa (13)
Acinetobacter baumannii complex	09	Acinetobacter lwofii (07) Acinetobacter junii (02)
Streptococcus pneumonia	04	Streptococcus pneumoniae (04)
Staphylococcus aureus	04	Staphylococcus aureus (04)
Escherichia coli	03	Escherichia coli (03)
Klebsiella pneumoniae	02	Klebsiella pneumoniae (02)
Yersinia pseudotuberculosis	00	Yersinia pseudotuberculosis (01)
Nocardia	01	Nocardia cyriacigeorgica (01)

Table.2. Frequent bacterial isolates and the percentage of strains susceptible to antibacterial (ophthalmic) agents (n=57).

Organisms (n)	(no of isolates)	Azm n (%)	E n (%)	Doxy n(%)	Cot n(%)	Cip n(%)	Levo n(%)	Ak n(%)	Gen n(%)	Tob n(%)	C n(%)	PBs n(%)	Va n(%)	Tetra n(%)
Coagulase negative Staphylococcus species	20	13 (65)	7 (35)	-	8 (40)	20 (100)	20 (100)	11 (55)	10 (50)	10 (50)	2 (10)	-	20 (100)	15 (75)
Pseudomonas aeruginosa	13	-	-	-	-	13(100)	13 (100)	4(30.7)	7 (53.8)	2 (15.4)	-	13(100)	-	-
Acinetobacter baumannii complex	09	-	-	3 (33.3)	3 (33.3)	3(33.3)	3 (33.3)	9 (100)	3 (33.3)	9 (100)	-	9 (100)	-	-

Streptococcus pneumoniae	04	4 (100)	4 (100)	4 (100)	4 (100)	-	4 (100)	-	-	-	4 (100)	-	4 (100)	4 (100)
Staphylococcus aureus	04	4 (100)	4 (100)		4 (100)	4 (100)	4 (100)	4 (100)	4 (100)	4 (100)	4 (100)	-	4 (100)	4 (100)
Escherichia coli	03	-	-	3 (100)	3 (100)	2 (66.6)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	-	3 (100)
Klebsiella pneumoniae	02	-	-	2 (100)	2 (100)	1 (50)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	-	2 (100)
Yersinia pseudotuberculosis	01	-	-	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	-	1 (100)
Nocardia	01	-	-	-	-	-	-	-	-	-	-	-	-	-

Azm- Azithromycin, E- Erythromycin, Doxy- Doxycycline, Cot – Trimethoprim-sulfamethoxazole, Cip – Ciprofloxacin, Levo – Levofloxacin, Ak – Amikacin, Gen – Gentamicin, Tob – Tobramycin, C – Chloramphenicol, PBs – Polymyxins, Va – Vancomycin, Tetra – Tetracycline

Discussion

Microbial keratitis is a very common infection in our part of the world and is an ophthalmic emergency requiring urgent attention. Immediate accurate identification of the causative agent and treatment is mandatory specially if certain vision-threatening outcomes are to be avoided. The aetiology of microbial keratitis may vary according to geographical locations. Most of the studies quote fungus as the leading cause followed by bacteria. [7-10] Here we have only included the bacterial agents leading to keratitis. Our culture positivity was 35.4% which is slightly lower compared to the other studies [11].

Gram stain is a very important tool in the diagnosis of bacterial keratitis, as treatment can be initiated based on the smear results, without waiting for the culture and sensitivity results. The initial empirical therapy can be started immediately and depending upon the sensitivity results, treatment can be changed later. In our case, both Gram-positive and Gram-negative bacteria contributed equally to the aetiology. This is in contrast to the other studies which reported a higher increasing trend of Gram-positive organisms over the Gram-negative organisms.

Moreover, it has been found that Gram-positive organisms are the commoner etiological agents of microbial keratitis. [12,13] The most common Gram-positive bacteria isolated were coagulase-negative *Staphylococcus* (35%), whereas *Pseudomonas* spp. (23%) was the most commonly isolated gram-negative bacteria. Our results are in concordance with studies conducted in North India where an eight-year analysis yielded similar organisms [14].

All these isolates were confirmed and speciated with the use of MALDI TOF-MS (Bruker Biotyper Microflex, MA, USA). Besides these some rare organisms like *Yersinia pseudotuberculosis* were also identified using it. The increasing number of Gram-negative organisms causing keratitis has been attributed to the overwhelming widespread use of contact lens in the general population.

Coagulase-negative *staphylococcus* in our study depicted 100% sensitivity to vancomycin and ciprofloxacin with variable sensitivity to erythromycin (35%) and gentamycin (50%). All the commonly used antibiotics showed very good sensitivity against *Streptococcus pneumoniae*. The sensitivity pattern for *Pseudomonas* spp., the most common isolated gram-negative bacteria, was less than 50% for all aminoglycosides tested in our analysis and is very shocking. Tobramycin a new generation aminoglycoside, showed the least efficacy (15%) against these organisms.

Our findings are totally in contrast to a study done in South India, where they found the sensitivity of the aminoglycosides to be more than 80%. [14] But they were found 100% sensitive to ciprofloxacin and polymyxins. Amongst other gram-negative bacteria, such as *E. coli* and *Klebsiella* spp., sensitivity to all aminoglycosides was extremely good, but they were found to be less sensitive to ciprofloxacin (66% and 50%). Similar results were also seen in the case of *Acinetobacter baumannii* complex, where levofloxacin and ciprofloxacin were less effective. Our findings are consistent with studies conducted in the rest of the country. [15]

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Our culture positivity was 35.4% which is slightly lower compared to the other studies. [11] Gram stain is a very important tool in the diagnosis of bacterial keratitis, as treatment can be initiated based on the smear results, without waiting for the culture and sensitivity results. The initial empirical therapy can be started immediately and depending upon the sensitivity results, treatment can be changed later. In our case, both Gram-positive and Gram-negative bacteria contributed equally to the aetiology. This is in contrast to the other studies which reported a higher increasing trend of Gram-positive organisms over the Gram-negative organisms. Moreover, it has been found that Gram-positive organisms are the commoner etiological agents of microbial keratitis [12,13].

The most common Gram-positive bacteria isolated were coagulase-negative *Staphylococcus* (35%), whereas *Pseudomonas* spp. (23%) was the most commonly isolated gram-negative bacteria. Our results are in concordance with studies conducted in North India where an eight-year analysis yielded similar organisms. [14] All these isolates were confirmed and speciated with the use of MALDI TOF-MS (Bruker Biotyper Microflex, MA, USA). Besides these some rare organisms like *Yersinia pseudotuberculosis* were also identified using it. The increasing number of Gram-negative organisms causing keratitis has been attributed to the overwhelming widespread use of contact lens in the general population.

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Conclusion

This study shows the rising trend in the number of the gram-negative bacteria causing microbial keratitis in the country. For starting the correct empirical therapy, knowledge about the local epidemiological and antibiotic resistance pattern is very crucial.

What does the study add to the existing knowledge?

This will help the clinician in having an idea and help them to start the patient on appropriate empirical therapy based on the gram stain smear results without waiting for the culture report.

Author's contribution

Dr. Ranjana Rohilla: Concept

Dr. Aroop Mohanty: Study design

Dr. Suneeta Meena: Manuscript preparation

Dr. Neelam Kaistha: Statistical analysis

Dr. Pratima Gupta: Study design

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