Microbial Profile of Corneal Scrapping Samples Received in Department of Microbiology in a Tertiary Care Hospital, Himachal Pradesh

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Abstract: *Corneal diseases are the major cause of blindness in developing countries. Keratitis defined as inflammation of cornea, which can be of infectious or non - infectious etiology. Bacteria, fungi, viruses or parasites all can cause infectious keratitis, and in developing countries bacterial and fungal etiology is more common. A hospital based retrospective analysis of microbiological profile and antibiotic resistance pattern of all corneal scrapping samples received in Department of Microbiology, Tanda was done for a period of 2 years, i. e. from April 2022 to April 2024. Out of total 52 samples received, total of 13 (25%) samples were culture positive. Out of 13 samples, 9 (17.31%) samples showed bacterial growth, 4 (7.69%) samples were positive for fungal etiology. Out of 9 positive samples for bacterial isolates, 3 samples showed growth of Staphylococcus aureus, 3 Pseudomonas species, 1 Escherichia coli, 1 Enterobacter species and 1 of Acinetobacter species. Out of 4 positive samples for fungal etiology, 2 showed growth of Fusarium species, 1 Aspergillus flavus and 1 Penicillium species. In gram positive bacterial isolates, Doxycycline (100%), Vancomycin (100%) and Linezolid (100%) were the most sensitive drugs followed by Cotrimoxazole (66.67%) and Levofloxacin (66.67%). In Non - Lactose Fermenting bacteria (NLF), Doripenem (100%) and Colistin (10%) were the most sensitive drugs followed by Ceftazidime (50%) and Cefepime (50%). In Enterobacteriaceae, only Ampicillin - Sulbactam (50%) showed resistance.*

Keywords: corneal diseases, keratitis, microbiological profile, antibiotic resistance, developing countries

1. Introduction

One of the major public health problems in developing countries is blindness. Further in developing countries, cataract and corneal diseases are the major causes of blindness.[1]

Cornea is transparent part of eyeball, comprising of 1/6th of anterior part of eyeball. Its transparent nature allows light to transmit, help to focus light rays and further protects eye from infective organisms, UV rays and other harmful substances in environment.[2]

Any disease of cornea often can leave permanent opacity, which can lead to impaired vision in patient and also can be very painful for patient. Patients generally present with pain, lacrimation, photophobia and varying degrees of diminution of vision.[2]

According to the World Health Organization, corneal diseases are among the major causes of vision loss and blindness in the world today, after cataract and glaucoma.[3] Keratitis is defined as any type of inflammation of cornea. Keratitis can either be caused by infectious or non - infectious etiology. Corneal ulceration is defined as loss of epithelium of cornea with inflammation in surrounding part of cornea. Infectious keratitis can lead to ulceration due to necrosis and pus formation.[2]

Infectious agents causing keratitis can be - bacterial, fungal, viral or parasitic. Viral etiology is more common cause of corneal ulcer in developed countries whereas in developing countries bacterial and fungal etiology is more prevalent. Most common bacterial agents involved are - gram positive bacteria - *Staphylococcus aureus, Streptococcus pneumoniae, Neisseria gonorrhoeae, Pseudomonas aeruginosa, Escherichia coli*. Among the fungal agents are the most common are *Aspergillus spp., Fusarium* spp. or *Candida albicans*.[2]

Contact lens wearing with poor hygiene or overnight use, trauma, steroid use, dry eyes, exposure to contaminated water, etc. are the most common risk factors for infective corneal ulcers.[2] Agriculture is the occupation of majority of Indian population, and hence ocular injury with vegetative matter is quite common. Further, hot and humid climate favors mycotic etiology.[3]

2. Methods

Study setting, design and study population
This is hospital based retrospective analysis of microbiological profile and antibiotic resistance pattern of all corneal scrapping samples received in Department of Microbiology, Tanda for a period of 2 years, i.e. from April 2022 to April 2024.

Corneal scrapings received for both bacterial and fungal culture from patients of all age groups having corneal ulcers, as requested by ophthalmologists were included in the study.

All samples received for culture are included in the study were processed according to standard microbiological guidelines. Antimicrobial susceptibility of bacterial isolates was done by Kirby Bauer disk diffusion method and the results were interpreted as per CLSI guidelines.

3. Results

Total of 52 samples were received for culture in Department of Microbiology during the stated period. Out of 52 samples received, 29 (55.76%) samples were from male patients and 23 (44.23%) were from female patients.

Number of samples received from patients in age group 40 - 60 and >60 years were 19 (36.53%) in both, followed by 11 (21.15%) in 20 - 40 years of age group and least from <20 years of age group which were 3 (5.76%) out of 52. Out of 19 samples received from >60 years of age group 7 were positive for infective etiology and only 3 were positive in 40 - 60 and 20 - 40 years of age group, respectively.
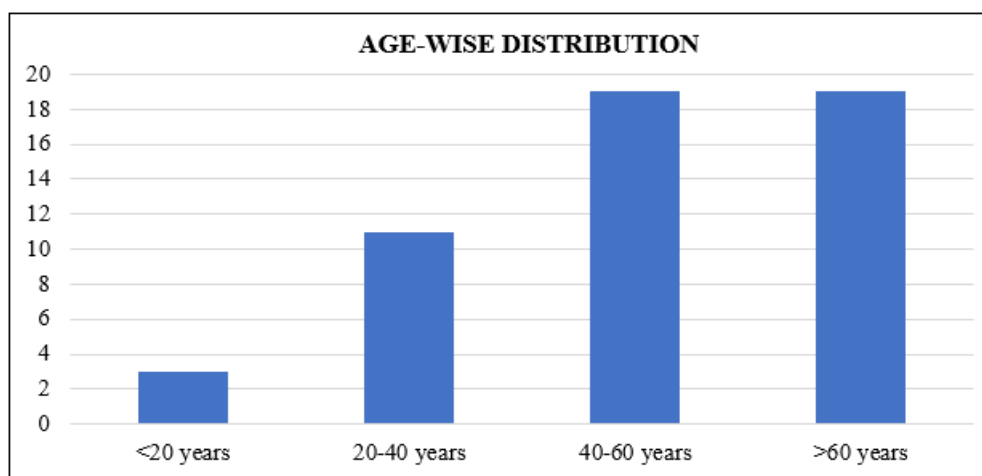
Microbiological Profile
Out of 52 samples received for culture, only 13 (25%) samples were culture positive. Out of culture positive samples, 9 (17.31%) samples showed bacterial growth, 4 (7.69%) samples were positive for fungal etiology and 39 (75%) samples were sterile.
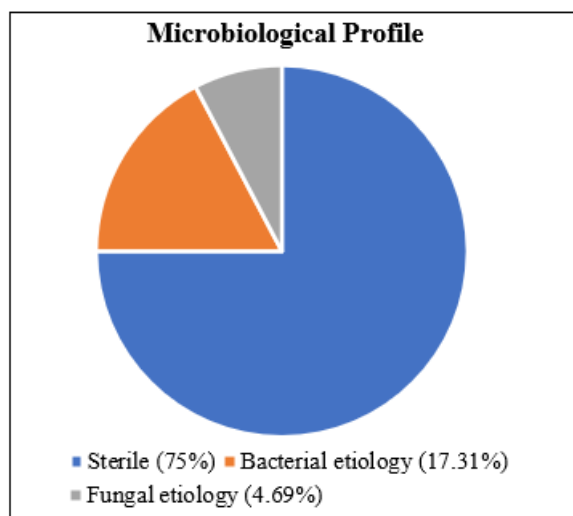
Distribution of Bacterial Isolates:
Out of 9 positive samples for bacterial isolates, 3 samples showed growth of *Staphylococcus aureus*, 3 *Pseudomonas aeruginosa*, 1 *Escherichia coli*, 1 *Enterobacter* species and 1 of *Acinetobacter* species.

Age Distribution of Patients for Aerobic Bacterial Culture Positivity:

AGE GROUP (years)	POSITIVE
<20	-
20 - 40	2
40 - 60	2
>60	5

Gender Distribution of Patients for Aerobic Bacterial Culture Positivity:

GENDER	POSITIVE
MALE	7
FEMALE	2

Organism	Number of isolates (%)
Staphylococcus aureus	3 (33.33%)
Pseudomonas aeruginosa	3 (33.33%)
Escherichia coli	1 (11.11%)
Enterobacter spp.	1 (11.11%)
Acinetobacter spp.	1 (11.11%)
Total	9

Antimicrobial Susceptibility Profile of Bacterial Isolates:

SENSITIVE/ RESISTANT									
GRAM POSITIVE BACTERIA									
	Meth	PG	Erythro	Clinda	Cotri	Doxy	Levo	Line	Vanco
Staphylococcus aureus	1/2	1/2	1/2	1/2	2/1	3/0	2/1	3/0	3/0

Meth: Methicillin, PG: Penicillin G, Erythro: Erythromycin, Clinda: Clindamycin, Cotri: Cotrimoxazole, Doxy: Doxycycline, Levo: Levofloxacin, Line: Linezolid, Vanco: Vancomycin.

SENSITIVE/RESISTANT									
GRAM NEGATIVE BACTERIA									
	Cefta	Cefe	Cipro	Tobra	Piptaz	Azt	Dori	Colistin	-
Pseudomonas	2/1	2/1	2/1	2/1	2/1	3/0	3/0	3/0	-
	Amp - S	Cefta	Cefe	Cipro	Tobra	Genta	Piptaz	Dori	Colistin
Acinetobacter	0/1	1/0	1/0	1/0	0/1	1/0	1/0	1/0	1/0
	Amp - S	Ceftria	Piptaz	Cefa	Genta	Cotri	Cipro	Doxy	Dori
E. coli	0/1	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0
Enterobacter	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0	1/0

Cefta: Ceftazidime, Cefe: Cefepime, Cefa: Cefazolin, Tobra: Tobramycin, Azt: Aztreonam, Cipro: Ciprofloxazin, Doxy: Doxycycline, Dori: Doripenem, Amp - S: Ampicillin - Sulbactam, Ceftria: Ceftriaxone, Piptaz: Piperacillin - Tazobactam, Cotri: Cotrimoxazole.

Distribution of Fungal Isolates
Out of 4 positive samples for fungal etiology, 2 showed growth of *Fusarium* species, 1 *Aspergillus flavus* and 1 *Penicillium* species. All fungal isolated were filamentous fungi and no yeast or yeast - like isolates were obtained.

Organism	Number of isolates (%)
Fusarium species	2 (50%)
Aspergillus flavus	1 (25%)
Penicillium species	1 (25%)
Total	4

Age distribution of patients for fungal culture positivity:

Age Group	Positive
<20	-
20 - 40	1
40 - 60	1
>60	2

Gender distribution of patients for fungal culture positivity:

Gender	Positive
Male	2
Female	2

4. Discussion

Out of 52 samples received, 13 (25%) samples showed growth. As majority of patients were referred from peripheral institutes to tertiary care centre and were already on empirical treatment, that could be the reason that most 39 (75%) of samples received for culture were sterile. Moreover, viral or parasitic causes, aerobic or fastidious bacterial agents could be the cause of corneal ulcers, which were not included in this study. Out of 13 culture positive samples, 9 (69.2%) showed growth of bacterial isolates and 3 (23.07%) showed growth of fungal isolates.

In present study, male to female ratio was 1.26: 1 as observed in Ahmedabad [5], Kashmir [6] and South India [7]. Occupation related injuries, like - while farming, is more common in adults could explain that maximum study subjects were in age group 40 - 60 and >60 years of group. Comorbidities like - hypertension, diabetes mellitus, impaired immunity due to complex and chronic illness leading to delayed healing and neglect by families is more common in elderly could explain that majority of culture positive samples (53.84%) belonged to >60 years of age.

Out of 13 culture positive specimen, bacterial etiology was more common than fungal as found in Ahmedabad [5]. Further, bacteriological profile showed that gram negative bacteria 6 (66.67%) were more prevalent than gram positive bacteria 3 (33.33%). Out of all gram - negative bacteria, Pseudomonas spp. was most common isolate 3 (33.33%) and Staphylococcus aureus was the only gram - positive bacteria isolated. Out of 3 isolates of Staphylococcus aureus, 1 isolate was MSSA and 2 isolates were MRSA.

In gram positive bacterial isolates, Doxycycline 3 (100%), Vancomycin 3 (100%) and Linezolid 3 (100%) were the most sensitive drugs followed by Cotrimoxazole 2 (66.67%) and Levofloxacin 2 (66.67%).

In Non - Lactose Fermenting bacteria (NLF), Doripenem 4 (100%) and Colistin 4 (10%) were the most sensitive drugs followed by 6 2 (50%) and 7 2 (50%). In Enterobacteriaceae, only Ampicillin - Sulbactam 1 (50%) showed resistance.

Predominant fungal isolate obtained was Fusarium spp.2 (50%), followed by Aspergillus flavus and Penicillium spp.1 (25%) each. No yeast form was isolated.

5. Conclusion

Males were more affected than females. Further, > 60 years of age group showed more culture - positivity. Bacterial ulcers were more common than fungal in infective etiology. Most common bacterial isolates were *Staphylococcus aureus* and

Pseudomonas spp. In fungal etiology, *Fusarium* spp. was found to major isolate.

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