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Research Article

Microbiological Profile and Their Antimicrobial Susceptibility in Infective Keratitis at Regional Eye Hospital, Visakhapatnam

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Abstract: Keratitis (corneal ulcer) is a leading cause of ocular morbidity and blindness worldwide especially in developing countries second only to cataract. It may be caused by bacteria, fungi, viruses and acanthamoeba. The aim of the study is to isolate, identify the bacteria and fungi in corneal scrapings from keratitis cases and to study their antibacterial and antifungal susceptibility patterns respectively. The material for the present study include corneal scrapings collected from 100 patients with clinical diagnosis of corneal ulcer with or without hypopyon attending Regional Eye Hospital, Visakhapatnam from September 2011 to September 2013. The corneal scrapings were processed by bacterial and fungal culture methods and microscopic examination by Gram's stain and KOH mount. Bacterial isolates were identified by standard biochemical methods and their susceptibility testing done by Kirby-Bauer disc diffusion method. Fungal isolates were identified by microscopic morphology and antifungal susceptibility testing was performed according to CLSI M 44-A for Candida sps and CLSI M51-A for the isolated moulds. Results showed higher prevalence of keratitis in 21 – 40 yrs age group. 73% culture positivity was observed which include 21 bacterial, 49 fungal and 3 mixed isolates. Pseudomonas aeruginosa (29.17%) was predominant bacterial isolate followed by Staphylococcus aureus (25%). Among 52 fungal isolates Fusariumsps. (36.54%) was predominant followed by Aspergillus sps(32.69%). Fungal isolates showed higher resistance to Fluconazole. To conclude, the study of microbial analysis and their susceptibility testing would greatly help in the specific treatment and management of keratitis cases.
Keywords: Infective keratitis, corneal scrapings, KOH mount, moulds, antifungal susceptibility testing

INTRODUCTION

Corneal ulcer is second leading cause of ocular morbidity and blindness worldwide especially in developing countries next to cataract [1, 2]. Considering the importance of corneal ulceration many studies have reported the prevalence of microbial pathogens [3, 4]. Quick and accurate identification of the causative micro-organisms and their antimicrobial susceptibility helps in specific treatment [4]. Antifungal susceptibility is not performed regularly and thusled to empirical treatment of fungal corneal ulcer. The choice of the antifungal agents to manage cases is becoming difficult due to development of resistance amongst the pathogenic fungi. The CLSI M 44-A and CLSI M- 51A documents described disc diffusion susceptibility testing for yeasts and non dermatophyte filamentous fungi respectively that can be used for routine diagnostic purposes [5, 6].

The objective of the present study was to isolate and identify the bacterial and fungal pathogensof corneal ulcer in corneal scrapings from keratitis patients and to study their antibacterial and antifungal susceptibility patterns.

MATERIALS AND METHODS

Corneal scrapings from hundred patients with the clinical diagnosis of corneal ulcer with or without hypopyon attending Regional Eye Hospital, Visakhapatnam from September 2011 to September 2013 were included in the study

All patients underwent thorough slit-lamp biomicroscopic examination & corneal scrapings were collected under aseptic conditions from leading edge and base of the ulcer by an ophthalmologist after instillation of 4% lignocaine drops, using a sterile No: 15 Bard Parker blade and inoculated on to the surface of blood agar, chocolate agar and Sabouraud's dextrose agar in a row of C shaped streaks (Fig. 1, 2) and into brain heart infusion broth [7]. Two smears were made for Gram's staining and KOH mount. Gram's stain was

examined for the presence of pus cells, microbes. 10% KOH mount was examined for the presence of fungal hyphae [7, 8] and microscopic examination report was immediately informed to the ophthalmologist.

All inoculated media were incubated aerobically. Microbial cultures were considered positive only if at least one of the following criteria were met [9-11].

- The growth of the same organism was demonstrated on two or more solid media on the C-streak; or there was semi confluent growth at the site of inoculation on one solid medium,
- The same organism was grown from repeated scraping,
- It was consistent with clinical signs,
- Smear results were consistent with cultures

The specific identification of bacterial pathogens was done using standard biochemical identification tests. Fungal isolates were identified by macroscopic and microscopic morphology (Fig. 3-5) using standard laboratory criteria [7, 9, 12].

Antibiotic sensitivity testing was done for all the bacterial isolates on Mueller – Hinton agar by the Kirby – Bauer disc diffusion technique. Blood agar was used for fastidious organisms [12].

Antifungal susceptibility testing was performed for isolates of Fusarium spp, Aspergillus spp according to CLSI M 51-A document [5] and for Candida spp CLSI document M44-Awas followed [6].The isolated moulds were sub cultured on to potato dextrose agar one week prior to testing. The mold stock inoculum suspensions were prepared from 7 day old cultures grown on potato dextrose agar and adjusted spectrophotometrically to optical densities ranged from 0.09 to 0.11 at 530nm wavelength. The entire surface of Mueller Hinton agar plate was inoculated with inoculum suspension using a sterile swab and antifungal disks were placed. The plates were incubated at 25^0C for 48 - 72hrs and zones of inhibition were observed (Fig. 7).

The isolated yeasts were grown on Sabouraud's Dextrose Agar for 24 hrs, inoculum was prepared in distilled water and adjusted to match the turbidity of 0.5 Mac Farlands standard using spectrophotometer set at 530 nm wavelength. Sterile applicator swab was moistened in that cell suspension and used to inoculate the surface of Mueller Hinton agar plate supplemented with 2% glucose and methylene blue (0.5µg/ml), and then antifungal discs were placed and incubated at 25° C for 24 hrs and observed for zones of inhibition. The antifungal discs used were Nystatin, Itraconazole, Ketoconazole, Amphotericin B, Clotrimazole and fluconazole.

RESULTS

58% of patients were males and 42% were females. Corneal ulcers showed a higher prevalence (52%) in the economically active age group of 21-40 years (Table 1). Culture positivity was obtained in 73% of cases. Pure bacterial growth was obtained in 21% of cases, pure fungal growth in 49% of cases and 3% showed mixed growth. 27% cases did not show any growth.

Among the 24 bacterial isolates, 16 (66.67%) were Gram positive bacteria and 8 (33.33%) were Gram negative bacteria. *Pseudomonas aeruginosa* (29.17%) was predominant isolate followed by *Staphylococcus aureus* (25%). Other bacterial isolates include *Staphylococcus epidermidis* (20.83%), *Streptococcus pneumoniae* (12.5%), *Corynebacterium spp* (8.34%) and *Klebsiella pneumoniae* (4.16%) (Table 2).

Table 1: Age wise distribution of corneal ulcer patients (n=100)

Age in years	Number of cases	Percentage
< 20	5	5 %
21 – 40	52	52 %
41 – 60	33	33 %
> 60	10	10 %

Table 2: Various bacterial isolates of the present study

Isolates (n=24)	Number	Percentage
Pseudomonas aeruginosa	7	29.17 %
Staphylococcus aureus	6	25 %
Staphylococcus epidermidis	5	20.83 %
Streptococcus pneumoniae	3	12.5 %
Corynebacterium spp.	2	8.33 %
Klebsiella pneumoniae	1	4.17 %
Total	24	100 %

Of the 52 fungal isolates predominant isolate was *Fusarium spp* with 19 isolates followed by *Aspergillus spp* with 17 isolates. *Candida spp* and *Curvularia spp* were 5.76% each, *Penicillium spp*

3.85%, *Pseudallesheria boydii*, *Cladosporium spp*, *Paecilomyces spp*, *Scopulariopsis spp*, *Epicoccum spp*, *Alternaria spp* 1.92% each. 3.85% of the isolates were unidentified. Out of the 17 isolates of Aspergillus spp, 7 were *Aspergillus fumigatus*, 6 were *Aspergillius flavus* and 4 were *Aspergillus niger* (Table 3).

Table 3: Distribution of various Fungal isolates (n=52)

Isolates	No.	Percentage
Fusarium spp.	19	36.54
Aspergillus spp.	17	32.69
Curvularia spp	3	5.77
Penicillium spp	2	3.85
Candida albicans	2	3.85
Candida tropicalis	1	1.92
Pseudallesheria boydii	1	1.92
Cladosporium spp	1	1.92
Paecilomyces spp	1	1.92
Scopulariopsis spp	1	1.92
Epicoccum spp	1	1.92
Alternaria spp	1	1.92
Un identified	2	3.85
Total	52	99.99

Table 4: Antibiotic sensitivity pattern of Gram positive isolates (n= 16)

Isolates	No. of strains	Drugs tested and No. of strains sensitive/resistant													
		Am		Ak		Mo		Of		Ox		Cf		Ctz	
		S	R	S	R	S	R	S	R	S	R	S	R	S	R
Staph. aureus	6	0	6	4	2	4	2	2	4	2	4	4	2	4	2
Staph. epidermidis	5	2	3	3	2	5	0	3	2	5	0	5	0	5	0
Strerp. pneumoniae	3	2	1	3	0	3	0	2	1	-	-	2	1	3	0
Corynebacterium spp	2	2	0	2	0	2	0	1	1	-	-	1	1	2	0

Am-Amoxycillin, Ak- Amikacin, Mo-Moxifloxacin, Of-Ofloxacin, Ox-Oxacilln, Cf- Cefazolin, Ctz-Ceftazidime, S-Sensitive, R – Resistant, (-) – not tested.

Among the Gram positive isolates *Staphylococcus aureus* is showing highest resistance to all the tested antibacterial drugs followed by *Staphylococcus epidermidis*. Both the isolated Gram negative bacilli were sensitive to Imipenem.

Table 5: Antibiotic sensitivity pattern of Gram negative bacterial isolates (n=8)

Isolates	No. of strains	Drugs tested and No. of strains sensitive/resistant													
		Gen		Ak		Mo		Of		Cf		Ctz		Imp	
		S	R	S	R	S	R	S	R	S	R	S	R	S	R
Pseudomonas aeruginosa	7	2	5	4	3	3	4	3	4	4	3	5	2	7	0
K. pneumoniae	1	0	1	1	0	1	0	0	1	0	1	1	0	1	0

Gen- Gentamycin, AK-Amikacin, Mo- Moxifloxacin, Of- Ofloxacin,Cf- Cefazolin, Ctz- Ceftazidime, Imp- Imipenem, S – Sensitive, R - Resistant

Table 6: Antifungal susceptibility pattern of fungal isolates:

Isolates	No. of strain	Drugs tested and no. of sensitive/ resistant											
		NS		IT		KT		AP		CC		FLU	
		S	R	S	R	S	R	S	R	S	R	S	R
Fusarium	19	19	0	17	2	14	5	15	4	13	6	6	13
Asp. fumigatus	7	7	0	6	1	6	1	7	0	4	3	4	3
Asp. flavus	6	5	1	6	0	5	1	5	1	4	2	2	4
Asp. niger	4	4	0	4	0	2	2	2	2	2	2	1	3
C. albicans	2	2	0	2	0	2	0	2	0	2	0	2	0
C. tropicalis	1	1	0	1	0	1	0	1	0	1	0	1	0

NS-Nystatin, IT- Itraconazole, KT- Ketaconazole, AP- Amphotericin B, CC- Clotrimazole, FLU-Fluconazole, S–Sensitive, R – Resistant.

All the fungal isolates were sensitive to Nystatin except one isolate of *Aspergillus flavus*. In the present study 57.14% of bacterial corneal ulcers and 28.57% of fungal ulcers responded to treatment and healed with corneal scar.

DISCUSSION

Bacterial and fungal isolation in the present study was 28.76% and 67.12% respectively. Culture negativity in the present study was in 27% of cases. High culture negativity of 78.64% was reported in the study of Geetakumari PV *et al*. [13].

In the present study Gram positive bacterial isolates accounted for 66.67% andGram negative isolates accounted for 33.33% and it coincides with findings of Geetakumari PV *et al*., [13]. UshaGopinathan *et al*., reported 66.2% of the bacterial isolates were Gram positive [14]. *Pseudomonas aeruginosa* was the predominant (29.17%) bacterial isolate in the present study and similar isolation was seen in Geetakumari PV *et al*. [13].

Table 7: Comparison of various Bacterial isolates with previous studies

Author	*Pseudomonas aeruginosa*	*Staph. aureus*	*Staph. epidermidis*	*Streptococcus pneumoniae*	*Corynebacterium species*	*Klebsiellapneumoniae*
Basak SK *et al*., (2005)West Bengal [1]	21.1	42.6	15.7	9.4	2.7	-
AartiTewari *et al*., (2012), Ahmedabad [2]	18.9	32.7	25.8	-	-	6.8
Bharathi MJ *et al*., (2002), South India [4]	18.03	3.87	17.4	37.5	4.15	1.08
Geetakumari PV *et al*., (2011), Kerala [13]	26.14	15.9	9.09	26.14	-	-
UshaGopinathan *et al*., (2009), Hyderabad [14]	12	5.3	32.5	13.9	14.5	0.4
Nada ALYousuf, (2009), Bahrain [15]	54	10	-	12	2	0.7
Present study (2011- 13) Visakhapatnam	29.17	25	20.83	12.5	8.34	4.16

Table 8: Fungal isolates in various studies

Author	*Fusarium spp*	*Aspergillus spp*	*Candida spp*	*Curvularia spp*	*Penicillium spp*	*Pseudalles boydii*	*Paecilomyces spp*	*Scopulariopsis spp*	*Epicoccum spp*	*Cladosporium spp*	*Alternaria spp*
AartiTewari *et al*., (2012), Ahmedabad [2]	22.5	35.4	12.9	16.1	-	-	-	-	-	-	-
UshaGopinathan *et al*., (2009), Hyderabad [14]	35.6	28.9	0.76	5.4	0.1	-	-	-	0.06	0.5	0.3
Jagdishchander *et al*., (2008), Chandigarh [16]	23.53	41.18	8.82	5.88	2.94	-	2.94	-	-	-	-
SumanSaha *et al*., (2009), West Bengal [17]	10.81	55.4	18.91	-	-	-	-	-	-	-	-
Reema Nath *et al*., (2011), Assam [18]	25	19	1.1	18.4	15.2	-	1.6	-	-	8.2	-
Present study (2011– 13), Visakhapatnam	36.54	32.69	5.77	5.76	3.85	1.92	1.92	1.92	1.92	1.92	1.92

In the present study, the predominant isolate was *Fusarium spp* accounting for 36.54%. This observation coincides with Usha Gopinathan *et al*. [14]. *Curvularia spp* were 5.77% of fungal keratitis cases in the present study and correlates with the observations of UshaGopinathan *et al*. (5.4%) & Jagadesh Chander *et al*. (5.88%) [14,16]. Aarti Tewari *et al*. & Reemanath *et al*. reported high incidence of Curvularia - 16.1% & 18.4% respectively [2, 18].

Fig. 1: Blood agar showing bacterial colonies

Fig. 2: Blood agar showing fungal colonies

Fig. 3 Fusarium species growth on SDA, LCB mount

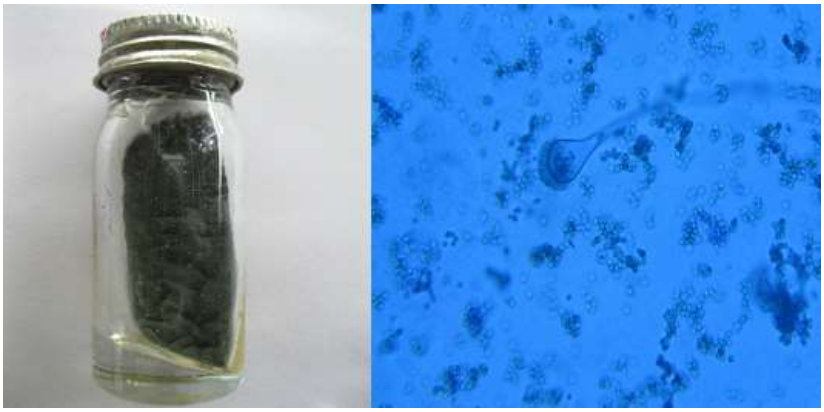

Fig. 4: *Aspergillus fumigatus* growth on SDA, LCB mount

Fig. 5: Curvularia species growth on SDA, LCB mount

Fig. 6: Antibiotic sensitivity testing of *Pseudomonas aeruginosa*

Fig. 7 Antifungal susceptibility testing for *Aspergillus species*

CONCLUSION

The study of microbial analysis of etiology of corneal ulcer would greatly help the practicing ophthalmologist in the management of infective keratitis. Because of increase of resistance to antimicrobial agents in pathogenic bacteria and fungi, susceptibility testing performed using commercially available media and discs following CLSI guidelines will be useful.

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