

Bacteriological profile of corneal ulcer with references to Antibiotic susceptibility in a tertiary care hospital in West Bengal

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Abstract: Over one year, 80 OPD patients with corneal ulcers were scraped from the margins & base of the anaesthetized cornea & smear prepared for Gram staining & 10% KOH preparation. Blood agar for both aerobic & anaerobic, Mac-Conkey Agar, Chocolate Agar were inoculated. Turbidity in brain heart infusion were identified by Gram Stain & subsequently sub-cultured in Mac-Conkey & blood agar. Anaerobic jar containing the blood agar plates were incubated at 37°C & examined after 48-72 hrs, & finally for 5 days & examined on alternative days before discarding. Further microbiological identification done as per standard protocol. All the isolated bacteria tested against different antimicrobial agents by standard disc diffusion method in accordance with CLSI guideline 2012. Out of 80 cases, corneal ulcer showed male preponderance (3:1) the highest no.19 (23.75%) cases were in the age group of 51-60 years. A total of 40 positive cultures were aerobes.12 specimens for anaerobic culture showed no growth. Among the isolates 19 (46.34%) were *Staphylococcus aureus*, 13 (31.7%) were CONS, 5 (12.19%) were *Pseudomonas*, 3 (7.31%) were *E. Coli* & 1 (2.43%) was *Klebsiella*. Gram positive cocci were maximally sensitive to Vancomycin, Tobramycin with highest resistance to Ciprofloxacin (55.87% sensitive). Gram negative isolates were maximally sensitive to Chloramphenicol & Moxifloxacin & resistant to Norfloxacin (44.44% sensitive). Routine microbiological examination of corneal ulcer is necessary to analyse & compare the changing trends in the microbial etiology & their susceptibility pattern to formulate a proper & appropriate antibiotic response against corneal ulcer.

Key words: Corneal ulcer, Aerobes, Antimicrobial agents.

I. Introduction :

Corneal blindness is a major public health problem in India & infections constitute the most predominant cause. Microbial infections & immunologic conditions like hypersensitivity, allergy can elicit inflammatory response in the cornea. Microbial pathogens can cause corneal damage directly or by release of toxins & enzymes or by activating the immune system. An intact epithelium acts as a barrier against most organism except *Neisseria gonorrhoeae*, *Corynebacterium diphtheriae*, *Haemophilus influenzae*. For all other bacteria, a breach in the epithelium is useful. Local risk includes ocular trauma, dry eye, corneal edema, or surgery. Some other pathogenic systemic disorder may also contribute to the invasion by bacteria.¹(Rao *et al*, 2000).

The microbial causes of keratitis varies considerably between continents & countries & also within countries² (Leck *et al*, 2001). The hallmark of treating keratitis is the prompt institution of appropriate antimicrobial therapy to minimize corneal scarring & visual loss³(Asbell *et al*, 1982).

Morbidity can be significantly reduced when the treatment modality is guided by the knowledge of the causative organism & it's antimicrobial susceptibility⁴(Sharma *et al*, 2000).

Aims of the study : The present study identified the bacteriological profile along with it's antimicrobial susceptibility pattern in corneal ulcer cases.

Methods & materials :

The cases for the present study were selected from 80 OPD patients presented with features of corneal ulcers from June 2012 to May 2013. An initial elaborate h/o & detailed clinical examination with special attention to area & density of ulceration, size of the epithelial defect, degree of stromal edema, sclera involvement, anterior chamber reaction, routine blood examination & microbiological profile was done.

Multiple scrapings taken by universal eye speculum from the margins as well as base of the anaesthetized cornea & smear prepared for Gram staining & 10% KOH preparation. For aerobic cultivation, all solid agar (Blood agar for both aerobic & anaerobic, Mac Conkey Agar, Chocolate Agar) were inoculated on the surface without cutting the agar & C-shaped inoculations were made at sites (multiple)⁵ Srinivasan *et al*,1994. Thioglycollate broth used for transport of anaerobic specimens. Any turbidity appeared in brain heart infusion were identified by Gram Stain & subsequently sub-cultured into Mac Conkey & blood agar. For anaerobic cultivation, the Anaerobic jar containing the blood agar plates were incubated at 37°C & examined after 48-72 hrs. Culture plates showing no growth were further incubated anaerobically for 5 days & examined on alternative days before discarding⁶ (Forbes *et al*, 2002). Characterization & identification of both aerobic &

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anaerobic organisms were done by studying the colony morphology , gram staining , motility , & biochemical reactions.

All the isolated bacteria were tested against different antimicrobial agents by standard disc diffusion method (Kirby Bauer Technique, 1996) in accordance with CLSI guideline 2012. For anaerobic bacteria , blood agar plates were incubated anaerobically at 37°C for 48 hours⁷. (Bauer *et al* 1996).

II. Results :

AGE GROUP IN YEARS	MALE (NO.)	FEMALE (NO.)	TOTAL (NO.) (%)
0-10	1	1	2 (2.50)
11-20	2	1	3 (3.75)
21-30	7	2	9 (11.25)
31-40	12	6	18 (22.5)
41-50	11	5	16 (20)
51-60	12	7	19 (23.75)
61-70	12	1	13 (16.25)
TOTAL	57 (71.25%)	23 (28.75%)	80 (100)

In the present study , out of 80 cases, corneal ulcer was seen in 57 (71.25%) males & 23(28.75%) females, the male to female ratio being 3:1. The highest no.19 (23.75%) cases were in the age group of 51-60 years followed by 18 (22.5%) in 31-40 years.

MONTH	NO.OF CASES	INCIDENCE IN PERCENTAGE
January	11	13.75
February	9	11.25
March	3	3.75
April	3	3.75
May	4	5
June	4	5
July	5	6.25
August	6	7.5
September	8	10
October	8	10
November	9	11.25
December	10	12.5
Total	80	100

It has been observed that keratitis occurred throughout the year , but a higher incidence was observed towards winter, mainly November (11.25%) cases, December (12.5%) cases , & January (13.75 %) cases.

Type of culture	Total No.	Culture positive		Culture negative	
		Number	Percentage	Number	Percentage
Culture for aerobes	80	40	50	40	50
Culture for obligate anaerobes	12	Nil	-	12	100

Out of 80 specimens tested, there was no growth of any bacteria in 40 specimens. The isolated in 40 positive cultures was aerobes. Anaerobic culture was done in 12 specimens, which showed no growth.

TYPE OF ISOLATES	NUMBER	PERCENTAGE
Multiple	1	2.5
Single	39	97.5
Total	40	100

The study showed that, out of 40 positive cultures 39 (97.5%) specimens showed single isolates & 1 (2.5%) cases showed growth of two isolates. Thus, total bacterial isolates were 41.

AEROBIC ISOLATES	NUMBER OF ISOLATES (n = 41)	PERCENTAGE
GRAM POSITIVE COCCI		
Staphylococcus aureus	19	46.34
CONS	13	31.7
GRAM NEGATIVE BACILLI	5	12.19
Pseudomonaas	3	7.31
E. Coli	1	2.43
Klebsiella		

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A total of 41 Gram positive aerobic bacteria were identified & followed, out of which 19 (46.34%) were Staphylococcus aureus, 13 (31.7%) were coagulase negative Staphylococci(CONS), 5 (12.19%) were Pseudomonas, 3 (7.31%) were E. Coli & 1 (2.43%) were Klebsiella.

ISOLATES	Of	Cf	C	Va	G	Tb	Mo	Nx	Ak
Staphylococcus aureus (19)	63.15%	57.89%	57.89%	100%	73.6% 14	100%	78.94%	68.42%	84.2%
CONS (13)	61.5%	53.84%	69.23%	100%	69.23% 9	100%	76.92%	76.92%	69.23%
Sensitive isolates (32)	62.32%	55.87%	63.56%	100%	71.42% 32	100%	77.93%	72.67%	76.72% 25
	20	18	20	32	23	32	25	23	25

All the aerobic Gram positive cocci were sensitive to Vancomycin, Tobramycin 100% each, to be followed by Moxifloxacin (77.93%), Amikacin (76.72%) & Gentamicin (71.42%) ..Highest resistance to Ciprofloxacin (only 55.87% sensitive) was seen in the Gram positive isolates.

ISOLATES	Of	Cf	C	G	Mo	Nx	Ak
Pseudomonas (5)	40%	80%	100%	60%	100%	40%	60%
	2	4	5	3	5	2	3
Klebsiella (1)	100%	0%	100%	100%	100%	0%	100%
	1	0	1	1	1	0	1
E. Coli (3)	100%	100%	100%	66.67%	100%	66.67%	100%
	3	3	3	2	3	2	3
Total sensitive isolates (9)	66.67%	77.78%	100%	66.67%	100%	44.44%	77.78%
	6	7	9	6	9	4	7

The anti microbial sensitivity of aerobic Gram negative isolates were 100% each for Chloramphenicol & Moxifloxacin.the next in the line were of Ciprofloxacin & Amikacin 77.78% each. Ofloxacin 66.67% & most resistance was Norfloxacin (only 44.44% sensitive .)

Of-ofloxacin , Cf- ciprofloxacin , C – Chloramphenicol , Mo- Moxifloxacin , Nx – Norfloxacin , Ak- Amikacin , G- Gentamicin Tb – Tobramycin , Va – Vancomycin ,

III. Discussion :

In the present study , the corneal ulcer found to be higher in males 57/80, (71.25%) than 23/80 (28.75%) females, the male to female ratio being 3:1. Study done by Ormerod *et al* (1987)⁸ showed that out of 210 patients, 149 patients were males & 61 were females, the male female ratio being 2.44:1.

The highest no.19 (23.75%) cases were in the age group of 51-60 years ,was noted in our study which corroborates with Cameron *et al* (2006)⁹ in Sydney Eye hospital, Sydney , where 41.9% (47/112 patients) were around the age group of 50 years.

It has been observed that keratitis occurred throughout the year , but a higher incidence was observed towards winter, mainly November (11.25%) cases, December (12.5%) cases , & January (13.75 %) cases which goes with the study done by Jones *et al*¹⁰, where 9 (30%) cases were prevalent during winter months & 8 (27%) cases during Autumn season.

Corneal ulceration is mainly of microbial etiology. In our study, out of 80 cases, 40(50%) cases were culture positive. Single bacterial isolates were seen in 39(97.5%) culture positive cases. Mixed (Polymicrobial) was seen in 1(2.5%) culture positive cases. Thus, total bacterial isolates in 80 patients were 41. All the bacterial isolates were aerobic.

Work done by Khanal *et al*¹¹ ,McKellar *et al*¹² in New Zealand, Vajpayee *et al*¹³ in North India, Upadhyay *et al*¹⁴ showed that 38.37%(33/86) , 58.6% (51/87) , 50%(18/36) , 63.20%(256/405) cases were culture positive respectively. Polybacterial growth detected in 15% (13/86) ,18.3% ,& 2.77% of cases in studies done by Khanal *et al*¹¹ , Upadhyay *et al*¹⁴ , & Vajpayee *et al*¹³ respectively. Almost all the bacterial isolates were aerobic except it were 3(.01%) in work done by Upadhyay *et al*¹⁴.

A total of 41 Gram positive aerobic bacteria were identified , out of which 19 (46.34%) were Staphylococcus aureus, 13 (31.7%) were coagulase negative Staphylococci,, 5 (12.19%). Cameron *et al*⁹ isolated 38% CONS & 11% Staphylococcus aureus, similarly, Tuft S J¹⁵ in London conducted a study where Gram Positive organisms accounted for 54% of isolates (Staphylococcus was 33.4%), In our study Gram Negatives were 5 (12.19%) Pseudomonas, 3 (7.31%) were E. Coli & 1 (2.43%) were Klebsiella whereas study done by Cameron *et al*⁹ found 21% pseudomonas, & 1.8% Klebsiella & Tuft S J¹⁵ found 24.8% pseudomonas , 6% E. coli & 4% Klebsiella.

All the aerobic Gram positive cocci were sensitive to Vancomycin, Tobramycin 100% each, to be followed by Moxifloxacin (77.93%) , Amikacin (76.72%) & Gentamicin (71.42%) . .Highest resistance to Ciprofloxacin (

only 55.87% sensitive) was seen in the Gram positive isolates. The anti microbial sensitivity of aerobic Gram negative isolates were 100% each for Chloramphenicol & Moxifloxacin.the next in the line were of Ciprofloxacin & Amikacin 77.78% each. Ofloxacin 66.67% & most resistance was Norfloxacin (only 44.44% sensitive .) Rao *et al*¹⁶, found 83.4% Vancomycin & 70.6% Gentamicin sensitivity for Gram positive cocci. Chloramphenicol, Ciprofloxacin, & Norfloxacin showed 71.7%,69.3% & 67.7% coverage in majority of corneal isolates. Golstein *et al*¹⁷ found Staphylococcus aureus resistant to Fluoroquinolones from 5.8% in 1993 to 35% in 1997 & for Ofloxacin from 4.7% to 35% in the same period. On the contrary, Trousdale *et al*¹⁸ showed 96% corneal isolates were susceptible to Ofloxacin & 92% were sensitive to Ciprofloxacin.

IV. Conclusion :

Routine microbiological examination of the patients with corneal ulcer is necessary so as to analyse & compare the changing trends in the microbial etiology & their susceptibility pattern. This can help in formulating a proper & appropriate antibiotic response against corneal ulcer.

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