Original Article

Types of organisms and *in-vitro* susceptibility of bacterial isolates from patients with microbial keratitis: A trend analysis of 8 years

Sujata Das, Ruchipriya Samantaray[1], Aparajita Mallick, Srikant K Sahu, Savitri Sharma[2]

Purpose: To report the distribution and trends of types of organisms and antibiotic susceptibility of the bacterial isolates obtained from patients with microbial keratitis. **Methods:** Microbiology records of culture-positive microbial keratitis that underwent a diagnostic corneal scraping and cultures were reviewed. Fungal, bacterial, and parasitic culture results and antibiotic susceptibility profile of bacteria were analyzed and comparisons were made between two halves of the study period (2007–2010 vs. 2011–2014). **Results:** A total of 3981 corneal scrapings were processed during the 8-year study period. Pathogen was recovered in culture in 1914 (48.1%) samples. Fungi, bacteria, and parasites constituted 38.7%, 60%, and 1.3% of the total isolates, respectively. The common fungal isolates were *Aspergillus* spp. (224/868, 25.8%) and *Fusarium* spp. (200/868, 23.0%), while common Gram-positive bacteria were *Streptococcus pneumoniae* (217/1125, 19.3%) and *Staphylococcus aureus* (185/1125, 16.4%), and common Gram-negative bacteria was *Pseudomonas* spp. (99/219, 45.2%). There was no significant difference in proportion of bacterial ($P = 0.225$) and fungal ($P = 0.421$) keratitis between the first half and second half of the study period. There was a significant increase in proportion of Gram-positive isolates ($P = 0.015$) [353/758 (46.6%) vs. 772/1482 (52.1%)] and decrease in proportion of Gram-negative organisms ($P = 0.044$) [88/758 (11.6%) vs. 131/1482 (8.8%)] in the recent years. In-vitro antibiotic susceptibility testing showed decrease in susceptibility to moxifloxacin for *Pseudomonas* spp. ($P = 0.016$) in recent years. **Conclusion:** Prevalence of fungal and bacterial keratitis has remained unchanged over the years. This study shows a significant increase in Gram-positive bacterial infection and decrease in Gram-negative bacterial infection of the cornea in the recent years.

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Microbial keratitis is an ophthalmic emergency which requires urgent attention. It is one of the leading causes of blindness.[1] It is commonly associated with contact lens wear, ocular trauma, surgery, and ocular surface diseases, but can also occur without any predisposing factor.[2–4] Accurate and rapid identification of the microorganism is required for successful treatment of the disease.[5–8] Smear and culture are considered to be the gold standards to identify the offending organisms and guide appropriate treatment.[9] The common organisms are bacteria, viruses, and fungi. The prevalence of different organisms varies in different geographical locations.[3,4]

With widespread use of broad-spectrum antibiotics, a corresponding change in the microbial spectrum and antibiotic susceptibility may occur.[10] Regional differences exist in terms of the organism isolated, their susceptibility, and resistance pattern.[11] Therefore, local epidemiological studies are required to provide evidence-based management of microbial keratitis. This study reviews the distribution and trends in types of organisms isolated and antibiotic susceptibility pattern of bacterial isolates from microbial keratitis during a period of 8 years (i.e. 2007–2014).

Methods

A retrospective review of microbiology records of all patients with microbial keratitis from a tertiary eye care center in eastern India who underwent a diagnostic corneal scraping for direct microscopy and cultures from January 2007 to December 2014 was conducted. Corneal scrapings with positive culture were included for analysis. The collected data included patient profile (age and gender), culture results, and antibiotic susceptibility profile of bacterial isolates. Culture results and antibiotic susceptibility profiles were analyzed. Cases of viral keratitis were not included in the analysis. Trend analysis of the data was done year-wise and in two blocks of 4 years each (i.e. 2007–2010 and 2011–2014).

As a part of the institute protocol, patients presenting with clinical features of microbial keratitis underwent slit

Cornea and Anterior Segment Services, L V Prasad Eye Institute, Bhubaneswar, [1]MKCG Medical College and Hospital, Brahmapur, Odisha, [2]Jhaveri Microbiology Centre, L V Prasad Eye Institute, Hyderabad, Telangana, India

Correspondence to: Dr. Sujata Das, Cornea Service, L V Prasad Eye Institute, Bhubaneswar - 751 024, Odisha, India. E-mail: sujatadas@lvpei.org

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lamp examination and corneal scrapings. Microbiological processing of the corneal scrapings included smear preparation for microscopy after Gram stain and potassium hydroxide with calcofluor white (KOH + CFW) mount. Corneal scrapings were also inoculated on appropriate media (5% sheep blood agar, chocolate agar, Sabouraud dextrose agar, potato dextrose agar, non-nutrient agar with *Escherichia coli*, thioglycolate broth, and brain heart infusion broth). All media were incubated aerobically at 37°C except chocolate agar (incubated in 5% CO_2 at 37°C). The media were observed for 14 days for any growth. Conventional Ziehl-Neelsen (ZN) stain and modified ZN stain using 1% H_2SO_4 were done whenever indicated. A culture was considered positive when there was growth of the same organism on two or more media, or confluent growth at the site of inoculation on one solid medium, or growth in one medium with consistent direct microscopy findings, or growth of the same organism on repeated corneal scrapings.

Cultured bacterial isolates were subjected to antimicrobial susceptibility testing to a range of antibiotics commonly used in the treatment of corneal ulcer. Antibiotic susceptibility was done by disc diffusion Kirby-Bauer method as per the Clinical and Laboratory Standards Institute (CLSI) guidelines, which classify organisms as susceptible, resistant, or intermediately susceptible to antibiotics. For data analysis in this study, organisms with intermediate susceptibility were grouped as susceptible.

The Chi-square test was used for the comparison of two proportions. A *P* value of ≤ 0.05 was regarded as evidence of significance.

Results

A total of 3981 corneal scrapings were taken during the 8 years of the study period. Pathogen was recovered in culture in 1914/3981 (48%) samples. More than one pathogen were isolated in 306/1914 (16%) samples. Mixed infections were reported in 165 cases (56 in 2007–2010 and 109 in 2011–2014). There was no significant difference between the two periods (*P* = 0.86). Results of direct microscopy were considered to determine the significance of culture and all instances of smear-positive, but culture-negative were excluded from the analysis. Corneal scrapings of patients of microsporidial keratitis with smear-positive for microsporidial spores were not included, as it does not grow in routine culture media.

Prevalence of distribution of different organisms

Distribution of fungi, bacteria, and *Acanthamoeba* (38.7%, 60%, and 1.3%, respectively) is shown in Table 1. The common isolates among fungi were *Aspergillus* spp. (224/868, 25.8%) and *Fusarium* spp. (200/868, 23%); in Gram-positive bacteria were *Streptococcus pneumoniae* (217/1125, 19.3%) and other *Streptococcus* spp. (55/1125, 4.9%), and *Staphylococcus aureus* (185/1125, 16.4%) and other *Staphylococcus* spp. (413/1125, 36.7%); and in Gram-negative bacteria was *Pseudomonas* spp. (99/219, 45.2%).

There was no significant difference in the proportion of bacterial [441/758 (58.2%) vs. 903/1482 (60.9%)] (*P* = 0.225) and fungal [303/758 (40.0%) vs. 565/1482 (38.1%)] (*P* = 0.421) keratitis between the first and the last 4 years. There was a significant increase in proportion of Gram-positive isolates [353/758 (46.6%) vs. 772/1482 (52.1%)] (*P* = 0.015) and decrease in proportion of Gram-negative organisms [88/758 (11.6%) vs. 131/1482 (8.8%)] (*P* = 0.044) in the recent years.

Overall the proportion of fungi (868/2240, 38.7%) and Gram-positive bacteria (1125/2240, 50.2%) was more compared to Gram-negative bacteria (219/2250, 9.8%) and *Acanthamoeba* (28/2240, 1.3%). The proportion of *Aspergillus* spp. and *Fusarium* spp. in the distribution of fungi shows an increasing trend [Fig. 1a]. Similarly, in Gram-positive bacteria, *Staphylococcus* spp. shows an increasing trend [Fig. 1b]. In Gram-negative bacteria, *Pseudomonas* spp. shows a decreasing trend during 2007–2011, while it is increasing steadily during 2012–2014 [Fig. 1c].

Susceptibility to antibiotics: Gram-positive organisms

The overall susceptibility of *S. pneumoniae* was > 90% to all the tested antibiotics, highest being to cefazolin (100%) and lowest to ciprofloxacin (204/213, 95.8%) [Table 2]. While the susceptibility of *S. aureus* to vancomycin was 100% it was the least to ciprofloxacin (96/180, 53.3%) [Table 3]. Higher proportion of *S. aureus* was susceptible to chloramphenicol compared to fluroquinolone. Methicillin resistance was found in 26/173 isolates (15%) of *S. aureus* (MRSA) by disc diffusion testing with cefoxitin. Although susceptibility to fourth generation fluroquinolone (gatiofloxacin and moxifloxacin) has decreased in the last 4 years, there was no significant difference in the susceptibility pattern of both the Gram-positive organisms (*S. pneumoniae* and *S. aureus*) in the last 4 years compared to the first 4 years.

Susceptibility to antibiotics: Gram-negative organisms

Overall, *Pseudomonas spp.* constitutes the largest proportion (99/219, 45.2%) of all Gram-negative isolates. The susceptibility tested against ciprofloxacin, ofloxacin, gatifloxacin, and moxifloxacin was: 92.6% (88/95), 93.6% (88/94), 94.8% (92/97), and 85.6% (77/90), respectively [Table 4]. Its overall susceptibility to gentamicin (74/92) and amikacin (44/53) was > 80%. There is a significant decrease in susceptibility to fourth generation fluroquinolone (moxifloxacin) in recent years (*P* = 0.016).

Discussion

The phenomenon of increasing antibiotic resistance is a matter of concern worldwide. The excessive and inappropriate systemic use of antibiotics is thought to be a leading factor for the emergence of antibiotic resistance. Literature has demonstrated that increasing use of antibiotics leads to development of resistant strains.[12,13] Therefore, periodic susceptibility surveys are important to detect emerging resistance patterns.

Prophylactic use of antibiotic has been associated with bacterial resistance. In ophthalmology, numerous reports have warned about indiscriminate use of ophthalmic antibiotics because it has been found to promote the emergence of antibiotic resistance.[14,15] Fluroquinolone is used widely as a monotherapy for presumed bacterial keratitis due to its broad-spectrum of action. However, increasing use of antimicrobial agents is responsible for development of resistance. Increasing in-vitro resistance of systemic and ocular isolates to fluroquinolones have been reported.[12,16]

We have included only culture-positive cases for analysis. Bacterial and parasitic keratitis constituted largest and least

Table 1: Year-wise distribution of microorganisms

	Year 2007	Year 2008	Year 2009	Year 2010	Year 2007-2010	Year 2011	Year 2012	Year 2013	Year 2014	Year 2011-2014	Year 2007-2014	Percentage
Fungi (%)	37 (12.2)	73 (24.1)	79 (26.1)	114 (37.6)	303 (100)	142 (25.1)	140 (24.8)	150 (26.5)	133 (23.5)	565 (100)	868	38.7
Gram-positive bacteria (%)	41 (11.6)	84 (23.8)	83 (23.5)	145 (41.1)	353 (100)	130 (16.8)	207 (26.8)	202 (26.2)	233 (30.2)	772 (100)	1125	50.2
Gram-negative bacteria (%)	20 (22.7)	16 (18.2)	24 (27.3)	28 (31.8)	88 (100)	23 (17.6)	38 (29.0)	35 (26.7)	35 (26.7)	131 (100)	219	9.8
Parasite (%)	2 (14.3)	2 (14.3)	4 (28.6)	6 (42.9)	14 (100)	2 (14.3)	4 (28.6)	6 (42.9)	2 (14.3)	14 (100)	28	1.3
Total (%)	100 (13.2)	175 (23.1)	190 (25.1)	293 (38.7)	758 (100)	297 (20.0)	389 (26.2)	393 (26.5)	403 (27.2)	1482 (100)	2240	100.0

Table 2: Year-wise susceptibility (%) of *Streptococcus pneumoniae*

	Year 2007 *n*=9	Year 2008 *n*=24	Year 2009 *n*=32	Year 2010 *n*=26	Year 2007-2010 *n*=91	Year 2011 *n*=28	Year 2012 *n*=39	Year 2013 *n*=40	Year 2014 *n*=19	Year 2011-2014 *n*=126	Year 2007-2014 *n*=217	*P*
Cefazolin	100.0	100.0	100.0	100.0	100.0	100.0	100.0	97.5	100.0	99.2	99.5	0.876
Vancomycin	85.7	100.0	93.8	100.0	96.5	100.0	100.0	100.0	100.0	100.0	98.6	0.123
Chloramphenicol	100.0	100.0	96.9	100.0	98.9	100.0	100.0	97.5	94.7	98.4	98.6	0.782
Ciprofloxacin	77.8	100.0	90.6	100.0	94.5	100.0	94.3	97.5	94.7	96.7	95.8	0.650
Ofloxacin	100.0	100.0	96.9	100.0	98.7	100.0	97.2	100.0	100.0	99.2	99.0	0.746
Gatifloxacin	66.7	100.0	90.3	100.0	94.1	100.0	92.1	100.0	100.0	97.6	96.2	0.336
Moxifloxacin	100.0	100.0	100.0		100.0	100.0	94.9	100.0	100.0	98.1	98.3	0.507

Table 3: Year-wise susceptibility (%) of *Staphylococcus aureus*

	Year 2007 *n*=3	Year 2008 *n*=9	Year 2009 *n*=13	Year 2010 *n*=28	Year 2007-2010 *n*=53	Year 2011 *n*=16	Year 2012 *n*=36	Year 2013 *n*=33	Year 2014 *n*=47	Year 2011-2014 *n*=132	Year 2007-2014 *n*=185	*P*
Cefazolin	100.0	100.0	100.0	92.9	96.2	100.0	91.7	97.0	89.4	93.2	94.1	0.663
Vancomycin	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	-
Chloramphenicol	100.0	100.0	84.6	85.7	88.5	87.5	94.4	84.8	87.2	88.6	88.6	0.813
Ciprofloxacin	66.7	25.0	61.5	53.6	51.9	31.3	53.1	66.7	53.2	53.9	53.3	0.933
Ofloxacin	100.0	57.1	53.8	78.6	70.0	56.3	79.4	81.8	60.5	70.6	70.5	0.923
Gatifloxacin	100.0	88.9	92.3	92.9	92.3	93.8	77.8	78.8	85.1	82.6	85.3	0.145
Moxifloxacin	-	-	66.7	64.3	63.2	56.3	66.7	53.1	55.3	58.0	59.2	0.736

Table 4: Year-wise susceptibility (%) of *Pseudomonas* spp.

	Year 2007 *n*=14	Year 2008 *n*=11	Year 2009 *n*=13	Year 2010 *n*=14	Year 2007-2010 *n*=52	Year 2011 *n*=7	Year 2012 *n*=12	Year 2013 *n*=12	Year 2014 *n*=16	Year 2011-2014 *n*=47	Year 2007-2014 *n*=99	*P*
Amikacin	66.7	100.0	100.0	-	77.8	75.0	90.9	83.3	81.3	83.7	82.7	0.626
Gentamicin	33.3	88.9	100.0	92.9	78.3	71.4	90.9	91.7	75.0	82.6	80.4	0.776
Ciprofloxacin	91.7	100.0	92.3	100.0	95.9	71.4	90.9	100.0	87.5	89.1	92.6	0.363
Ofloxacin	84.6	100.0	100.0	100.0	96.0	71.4	90.9	100.0	92.9	90.9	93.6	0.553
Gatifloxacin	92.3	100.0	100.0	100.0	98.0	71.4	91.7	100.0	93.8	91.5	94.8	0.312
Moxifloxacin	90.0	100.0	91.7	100.0	95.3	71.4	83.3	75.0	75.0	76.6	85.6	0.016

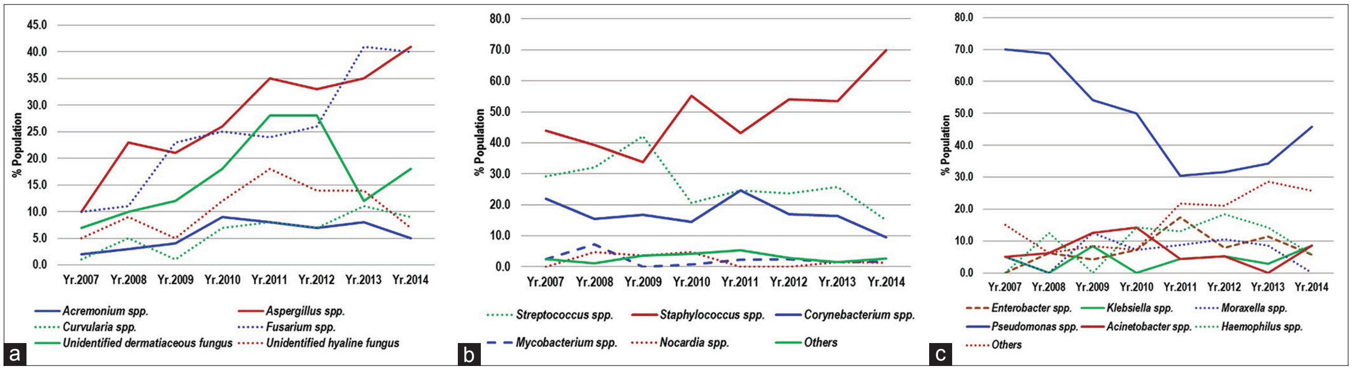

Figure 1: (a) Year-wise distribution of fungi. (b) Year-wise distribution of Gram-positive bacteria. (c) Year-wise distribution of Gram-negative bacteria

proportion, respectively. Our result showed 48.1% culture positivity. This is slightly lower compared to other studies.[17,18] This might be due to inclusion of microsporidial keratitis, which represents a major group of culture-negative microbial keratitis in our institute.[19] Microsporidial spores were confirmed by smear examination and they do not grow in conventional culture media. During the study period, 464 microsporidia cases were present. The lower rate of positive culture also might be due to culture of corneal scraping in all cases irrespective of prior medication and size.

The etiological agents for microbial keratitis vary in different geographical locations. Fungal keratitis is common in tropical and subtropical region. It accounts for 30–50% cases of microbial keratitis in developing countries.[20–23] Filamentous fungus is most commonly reported from patients in the tropical region.[3,20,21] Our findings correlate with the earlier studies published from India and other tropical countries.[3,20,21,24,25]

Gram-positive organism has been reported to be commoner etiological agent of microbial keratitis compared to Gram-negative organism,[2,17,26] *Staphylococcus* spp. and *Stereptococcus* spp. being most commonly isolated pathogens, which is similar to our study. In our study, Gram-positive organisms have in fact increased significantly in recent years. Correspondingly, Gram-negative organisms have decreased. Amongst Gram-positive organisms, *Staphylococcus* spp. have increased significantly ($P = 0.0003$) in the last 4 years. In the same period, *Pseudomonas* spp. have decreased significantly ($P = 0.001$), whereas other Gram-negative organisms are more or less stable. Pandita *et al.* have reported non-significant rise of Gram-positive organism in their 10 years' experience.[17] Alexndrakis *et al.* have described increased incidence of keratitis due to *S. aureus* and decreasing trend with *Pseudomonas aeruginosa*.[27] In contrast, Lichtinger *et al.* have reported an increase in Gram-negative and decrease in Gram-positive organism.[28] They have attributed this to increased use of contact lens. The increased trend of Gram-positive organisms in our study may be attributable to increasing number of patients with ocular surface problems and keratoplasties.

There are reports mentioning about rise in MRSA from different parts of the world.[26,28–30] Asbell *et al.* have reported an increase in the proportion of MRSA among *S. aureus* ocular infection from 29.5 to 41.6% in 5 years' time.[29] Lichtinger *et al.* have described a trend toward increasing laboratory resistance to methicillin from 28% during first 4 years to 38.8% in last 3 years.[28] In our study, MRSA is less compared to other reported studies. In the past, we had reported methicillin resistance in 7.8% cases with *S. aureus* ocular infection.[31]

Gradual increase of resistance of *S. aureus* to fluroquinolone has been reported.[27] In our series, susceptibility to cefazolin and vancomycin was better compared to fluoroquinolones [Table 3]. Although, there is decrease in susceptibility to gatifloxacin and moxifloxcacin for *S. aureus*, the pattern is stable for *S. pneumoniae*. An interesting observation was resistance of *Pseudomonas* spp. to moxifloxacin that has increased in recent years [Table 4]. This might be due to widespread use of moxifloxacin for various ocular infections as well as prophylactic use.

This study shows a significant increase in Gram-positive bacterial infection and decrease in Gram-negative bacterial infection of the cornea in the recent years. The Gram-positive organisms did not show a significant shift in their susceptibility to fluoroquinolones over the 8-year study period.

Conclusion

This study shows a significant increase in Gram-positive and decrease in Gram-negative bacterial infection of the cornea in the recent years. The Gram-positive organisms did not show a significant shift in their susceptibility to fluoroquinolones.

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Conflicts of interest

There are no conflicts of interest.

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