Original Article

Antibiotic susceptibility pattern of bacterial isolates from microbial keratitis in North and Central India: A multi centric study

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Purpose: This study was conducted to examine microbiological profile with their antibiotic sensitivity in cases of bacterial keratitis in north and central India to ensure appropriate use of antibiotics. **Methods:** The microbiology laboratory records of 228 patients with culture-proven bacterial keratitis from 1st January to 31st December 2019 were analyzed. Cultured bacterial isolates were subjected to antimicrobial susceptibility testing to antibiotics commonly used in the treatment of corneal ulcer. Chi-squared or Fisher's exact test were applied to check the significance of difference between the susceptibility levels of antibiotics. **Results:** The prevalence of *Staphylococcus aureus* and *Pseudomonas aeruginosa*–induced keratitis was higher in northern India, whereas that by *Streptococcus pneumoniae* was more prevalent in central India. In central India, 100% of *S. pneumoniae* isolates were found to be sensitive to ceftriaxone compared to 79% in northern India ($P = 0.017$). In comparison to 67% of isolates from north India, 15% of *S. aureus* isolates from central India were found to be sensitive to ofloxacin ($P = 0.009$). Similarly, 23% of isolates from central India were found sensitive to amikacin compared to 65% of isolates from north India ($P = 0.012$). *P. aeruginosa* isolates from central India were found to be sensitive to ceftazidime in 63% of cases compared to 21% of isolates from north India ($P = 0.034$). **Conclusion:** Prevalence of bacteria and their susceptibility to antibiotics are not uniform across geography. Vancomycin remained the most effective drug in all gram-positive coccal infections. *S. aureus* susceptibility to amikacin was significantly greater in north India. *P. aeruginosa* showed less susceptibility as compared to previous reports.

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Bacterial keratitis is one of the common causes of ocular morbidity.[1,2] The identification of bacterial pathogens and their screening for antibiotic sensitivity is crucial to initiate prompt antimicrobial therapy to save the eye of a keratitis patient. The emergence of antibiotic resistance in bacterial pathogens is becoming a serious public health concern.[3] Recent studies from India have reported emergence of antimicrobial resistance in ocular infections.[4] Geographical and temporal variations among antibiotic sensitivity patterns of different bacterial pathogens have been observed in previously reported studies from India and China.[4–6] These studies highlight the inter-individual sensitivity of bacterial pathogens to different antibiotics that vary according to species identified and antibiotics tested. Injudicious use of antibiotics in communities is one of the major contributing factors toward emergence of antibiotic resistance.

The periodic and area-wise reporting of microbiological profile with their antibiotic sensitivity is critical to ensuring appropriate use of antibiotics. This prompted the authors of the current study to review and report antibiotic sensitivity of bacterial pathogens causing keratitis, identified during January to December 2019. It is a collaborative effort of four tertiary eye care institutes having dedicated cornea and microbiology facilities. Two of them are located in north India and the other two are in central India. This report discusses the variation of antibiotic sensitivity among these two geographical regions of India with vastly different climates and population. The results of this study facilitate an understanding of appropriate

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and prudent use of antibiotics for the treatment of bacterial keratitis in these regions.

Methods

This retrospective review of laboratory records of patients with microbial keratitis was conducted at four tertiary eye care institutes in central and north India. All the participating centers are part of an eye consortium, which was formed to allow the development of evidence-based and consensus-led protocols through consistent and robust big data from eye care institutes in India. The study was approved by the Institutional Ethics Committee of all the participating institutes and adhered to the principles of the Declaration of Helsinki.

The microbiology laboratory records of all consecutive patients with culture-proven bacterial keratitis from 1st January to 31st December 2019 were included. The records of patients with co-existing endophthalmitis or mixed infections with fungi, viral or amoebae were excluded. Data related to demographic characteristics, types of bacteria species, and antibiotic susceptibility pattern were analyzed. The diagnostic work-up of microbial keratitis in the four institutes followed a common protocol that included detailed history-taking, slit-lamp examination, corneal scrapings, microbiological tests, patency of nasolacrimal duct, and random blood sugar evaluation.

Corneal scrapings were performed at the slit-lamp under topical anesthesia with 0.5% proparacaine eye drops. The corneal scrapings were used to prepare smears on sterile glass slides for direct microscopy with Gram stain and 10% potassium hydroxide and calcofluor white mount. Corneal scrapings were also directly inoculated in 5% sheep blood agar, chocolate agar, Sabouraud dextrose agar, potato dextrose agar, non-nutrient agar with *Escherichia coli* overlay, thioglycolate broth, and brain heart infusion broth. All media were incubated aerobically under the appropriate temperature. The media were observed for 14 days for any growth. Conventional Ziehl-Neelsen (ZN) stain and modified ZN stain using 1% H_2SO_4 was done whenever indicated. A culture was considered positive when there was growth of the same organism on two or more media, confluent growth at the site of inoculation on one solid medium, 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 against a range of antibiotics commonly used in the treatment of corneal ulcer. Antibiotic susceptibility was done using the Kirby–Bauer disc diffusion method as per the Clinical and Laboratory Standards Institute (CLSI) guidelines, which classify organisms as susceptible, resistant, or intermediately susceptible to antibiotics (Annexure 1). One institute had used VITEK analysis for antibiotic susceptibility and species identification which also gave reports in the same format. For this study, an antibiotic was labeled resistant if the zone of inhibition was categorized as intermediate or resistant. All laboratory methods were performed under standard protocols presented in annexure I.

Statistical analysis

Susceptibility percentages were presented only for those antibiotics that were tested at least for five individual bacterial isolates during the study period. Data related to susceptibility patterns are represented as a proportion with a 95% confidence interval (CI). For ease of data interpretation, antibiotic susceptibility of a bacterial isolate was categorized as high (>90%), moderate (>50% to <90%) and low (<50%). Individual susceptibility of every isolate against all antibiotics are presented in tables. For geographical comparison of antibiotic susceptibility, two groups were made: central India and north India. Chi-squared or Fisher's exact test were applied to check the significance of difference between the susceptibility levels of antibiotics across the geography. A forest plot was drawn to represent the susceptibility of different antibiotics against three bacteria of ocular importance. The statistical analysis was performed using R version 4.0.5. A two-tailed *P* value of less than 0.05 was considered statistically significant.

Results

The laboratory records of 228 patients with corneal ulcers who had bacterial growth on culture media during January to December 2019 were included in the analysis. The samples were obtained from 135 (59%) males and 93 (41%) females with a mean age of 51 ± 19.61 (2–91) years. Six percent of the patients were below 15 years, 23% between 16 and 39 years, 30% between 40 and 59 years, and 41% were above 60 years old. Forty-seven percent of the patients were from rural areas and 53% were from urban. The Rural urban ratio was much higher in the central zone (71:29) as compared to the north.

Microscopic examination of smears of the corneal scraping revealed 72.4% gram-positive cocci, 18.4% gram-negative bacilli, 7.5% gram-positive bacilli, 0.4% gram-negative cocci, and 1.3% mixed gram-positive cocci and gram-negative bacilli. The proportions significantly varied between the north and central zone ($P < 0.001$). Table 1 shows the distributions of different classifications of bacteria in the north and central zone. This observation can be a guide for choosing first-line therapy of drugs for general physicians practicing in these geographical areas who do not have access to bacterial culture facilities. The most frequent bacterial isolate was *Staphylococcus aureus* (88, 38.1%) followed by *Streptococcus pneumoniae* (64, 27.7%) and *Pseudomonas aeruginosa* (43, 18.6%). The distribution of all bacterial isolates is presented in Table 2. There was a significant variation in the prevalence of bacteria species between north and central India. Bacterial species more prevalent in north India were *S. aureus* (46.8%), *P. aeruginosa* (21.8%), while *S. pneumoniae* and *Bacillus* spp. were more prevalent in central India.

The distribution of antibiotic sensitivity of selected bacterial isolates between central and north India are presented in Table 3. The sensitivity patterns of the common isolates have been represented in the forest plot described in Fig. 1. The susceptibility pattern of different antibiotics against three commonest bacteria are summarized below.

Streptococcus pneumoniae

More than 90% of the *Streptococcus pneumoniae* isolates were found to be susceptible to vancomycin (95.1%), cefoxitin (93.3%), ceftriaxone (92%), and cefazolin (91.6%). The lowest susceptibility of *S. pneumoniae* was reported to amikacin (20.9%). The isolates were moderately susceptible (>50% to < 90%) to cefuroxime (89.3%), chloramphenicol (88.5%), piperacillin (78.5%), ofloxacin (77.5%), moxifloxacin (73%), ceftazidime (61%), gatifloxacin (53.3%), and

Table 1: Results of Microscopic Examination of Smears of Corneal Scraping

Bacteria Type	North India	Central India	Total	*P*
Gram-positive bacilli	0.7% (1)	21.3% (16)	7.5% (17)	<0.001 (Fisher's exact test)
Gram-positive cocci	75.2% (115)	66.7% (50)	72.4% (165)	
Gram-negative bacilli	21.6% (33)	12% (9)	18.4% (42)	
Gram-negative cocci	0.7% (1)	0% (0)	0.4% (1)	
Gram-positive cocci + Gram-negative bacilli	1.9% (3)	0% (0)	1.3% (3)	
Total	100% (153)	100% (75)	100% (228)	

Table 2: Frequency Distribution of Bacterial Isolates

Bacterial Isolates	North India	Central India	Total Sample	*P*
Staphylococcus aureus	46.8% (73)	20% (15)	38.1% (88)	<0.001
Streptococcus pneumoniae	20.5% (32)	42.7% (32)	27.7% (64)	
Pseudomonas aeruginosa	21.8% (34)	12% (9)	18.6% (43)	
Bacillus	0% (0)	14.7% (11)	4.8% (11)	
Corynebacterium spp.	2.6% (4)	0% (0)	1.7% (4)	
Klebsiella spp.	2.6% (4)	0% (0)	1.7% (4)	
Nocardia spp.	0% (0)	5.3% (4)	1.7% (4)	
Kocuria spp.	0% (0)	4% (3)	1.3% (3)	
Neisseria spp.	1.3% (2)	0% (0)	0.9% (2)	
Serratia spp.	1.3% (2)	0% (0)	0.9% (2)	
Escherichia coli	1.3% (2)	0% (0)	0.9% (2)	
Mycobacterium spp.	1.3% (2)	0% (0)	0.9% (2)	
Moraxella spp.	0.7% (1)	0% (0)	0.4% (1)	

ciprofloxacin (51.6%). Low susceptibility (<50%) of isolates was reported in the case of tobramycin (41.6%). In central India, 100% of *S. pneumoniae* isolates were found to be sensitive to ceftriaxone compared to 79% of isolates from north India ($P = 0.017$). Although in central India 97% of *S. pneumoniae* isolates were susceptible to cefuroxime compared to 78% of isolates in north India, the difference was only statistically borderline significant ($P = 0.063$). The susceptibility of *S. pneumoniae* isolates were similar in both geographical regions for all other antibiotics tested [Table 3].

Staphylococcus aureus

More than 90% of the *S. aureus* isolates were found to be susceptible to vancomycin (95.1%). The lowest susceptibility of *S. aureus* was to ceftazidime (19.7%). The isolates were moderately susceptible (>50% to <90%) to cefazolin (89.4%), cefoxitin (75%), chloramphenicol (65.3%), amikacin (58.3%), tobramycin (55.3%), and cefuroxime (53.1%). Low susceptibility (<50%) of isolates was reported in the case of piperacillin (46.6%), ofloxacin (45.1%), ceftriaxone (43.7%), moxifloxacin (40.9%), gatifloxacin (30.4%), and ciprofloxacin (27.9%). In central India, 15% of *S. aureus* isolates were found to be sensitive to ofloxacin compared to 67% of isolates from north India ($P = 0.009$). Similarly, 23% of isolates from central India were found to be sensitive to Amikacin compared to 65% of isolates from north India ($P = 0.012$). The sensitivity of *S. aureus* was similar in both geographical regions for all other antibiotics tested [Table 3].

Pseudomonas aeruginosa

The highest susceptibility of *P. aeruginosa* isolates was reported to be to ofloxacin (66.6%) and the lowest to ceftriaxone (9.6%). More than 90% susceptibility of *P. aeruginosa* isolates was not reported to any antibiotics tested (at least for 5 isolates). The isolates were moderately susceptible to tobramycin (64.8%), amikacin (62.7%), ciprofloxacin (57.1%), piperacillin (51.3%), and gatifloxacin (50%). Low susceptibility of *P. aeruginosa* was reported to be to moxifloxacin (47.3%), ceftazidime (29.2%), vancomycin (25%), chloramphenicol (20.5%), cefuroxime (5.6%), and cefazolin (10%). In central India, 63% of *P. aeruginosa* isolates were found to be sensitive to ceftazidime compared to 21% of isolates from north India ($P = 0.034$). The sensitivity of *P. aeruginosa* were similar in both geographical regions for all other antibiotics tested.

Discussion

Periodic reporting of sensitivity profiles of causative organisms of bacterial keratitis helps clinicians in choosing an effective therapy in a geographic region. This is the first study that has compared the bacterial sensitivity of bacterial keratitis pathogens in north and central India. In this study, gram-positive cocci accounted for 72.4% of total isolates of bacterial keratitis. This is similar to previously reported studies from India and other countries.[5,7,8] The central zone recorded a higher prevalence of gram-positive bacteria. The most frequent isolate was *S. aureus* followed by *S. pneumoniae* and *P. aeruginosa*. *S. aureus* was also identified as the most prevalent gram-positive and *P. aeruginosa* as the most prevalent gram-negative bacteria in previously reported studies.[5,7,8] However, in this study a difference in distribution of these isolates was reported between north and central India. *S. aureus* and *P. aeruginosa* were more prevalent in the north, whereas

Table 3: Susceptibility Pattern of Identified Bacterial Isolates

Antibiotic	Overall	North India	Central India	*P* (Fisher's Exact Test)
Streptococcus pneumoniae				
Vancomycin	95% (87%-98%, *n*=62)	100% (89%-100%, *n*=30)	91% (76%-97%, *n*=32)	0.239
Cefazoline	92% (65%-99%, *n*=12)	92% (65%-99%, *n*=12)	0% (0%-0%, *n*=0)	
Ceftriaxone	92% (81%-97%, *n*=50)	79% (57%-91%, *n*=19)	100% (89%-100%, *n*=31)	0.017
Cefuroxime	89% (77%-95%, *n*=47)	78% (55%-91%, *n*=18)	97% (83%-99%, *n*=29)	0.063
Chloramphenicol	89% (78%-94%, *n*=61)	81% (64%-91%, *n*=31)	97% (83%-99%, *n*=30)	0.104
Ofloxacin	78% (62%-88%, *n*=40)	75% (47%-91%, *n*=12)	79% (60%-90%, *n*=28)	1.000
Moxifloxacin	73% (61%-82%, *n*=63)	66% (48%-80%, *n*=32)	81% (64%-91%, *n*=31)	0.257
Gatifloxacin	53% (41%-65%, *n*=60)	45% (29%-62%, *n*=31)	62% (44%-77%, *n*=29)	0.208
Ciprofloxacin	52% (39%-64%, *n*=62)	41% (26%-58%, *n*=32)	63% (46%-78%, *n*=30)	0.083
Tobramycin	42% (29%-56%, *n*=48)	37% (19%-59%, *n*=19)	45% (28%-62%, *n*=29)	0.766
Amikacin	21% (13%-33%, *n*=62)	16% (7%-32%, *n*=32)	27% (14%-44%, *n*=30)	0.357
Staphylococcus aureus				
Vancomycin	95% (88%-98%, *n*=83)	96% (88%-98%, *n*=68)	93% (70%-99%, *n*=15)	0.557
Cefazoline	89% (69%-97%, *n*=19)	89% (69%-97%, *n*=19)	0% (0%-0%, *n*=0)	
Chloramphenicol	65% (54%-75%, *n*=75)	64% (52%-74%, *n*=69)	83% (44%-97%, *n*=6)	0.658
Amikacin	58% (48%-68%, *n*=84)	65% (53%-75%, *n*=71)	23% (8%-50%, *n*=13)	0.012
Tobramycin	55% (43%-67%, *n*=65)	60% (46%-72%, *n*=52)	38% (18%-64%, *n*=13)	0.218
Cefuroxime	53% (41%-65%, *n*=64)	57% (43%-69%, *n*=51)	38% (18%-64%, *n*=13)	0.352
Ofloxacin	45% (29%-62%, *n*=31)	67% (44%-84%, *n*=18)	15% (4%-42%, *n*=13)	0.009
Ceftriaxone	44% (32%-56%, *n*=64)	45% (32%-59%, *n*=51)	38% (18%-64%, *n*=13)	0.761
Moxifloxacin	41% (31%-52%, *n*=83)	41% (30%-52%, *n*=71)	42% (19%-68%, *n*=12)	1.000
Gatifloxacin	30% (22%-41%, *n*=82)	35% (25%-47%, *n*=69)	8% (1%-33%, *n*=13)	0.096
Ciprofloxacin	28% (20%-38%, *n*=86)	32% (23%-44%, *n*=71)	7% (1%-30%, *n*=15)	0.057
Pseudomonas aeruginosa				
Colistin	97% (86%-100%, *n*=37)	97% (84%-99%, *n*=31)	100% (61%-100%, *n*=6)	1.000
Ofloxacin	67% (42%-85%, *n*=15)	67% (35%-88%, *n*=9)	67% (30%-90%, *n*=6)	1.000
Tobramycin	65% (49%-78%, *n*=37)	61% (44%-76%, *n*=31)	83% (44%-97%, *n*=6)	0.395
Amikacin	63% (48%-76%, *n*=43)	56% (39%-71%, *n*=34)	89% (57%-98%, *n*=9)	0.121
Ciprofloxacin	57% (42%-71%, *n*=42)	55% (38%-70%, *n*=33)	67% (35%-88%, *n*=9)	0.708
Pipercillin	51% (36%-67%, *n*=37)	43% (27%-61%, *n*=30)	86% (49%-97%, *n*=7)	0.090
Gatifloxacin	50% (35%-65%, *n*=38)	52% (35%-67%, *n*=33)	40% (12%-77%, *n*=5)	1.000
Moxifloxacin	47% (32%-63%, *n*=38)	50% (34%-66%, *n*=32)	33% (10%-70%, *n*=6)	0.663
Ceftazidime	29% (18%-44%, *n*=41)	21% (11%-38%, *n*=33)	63% (31%-86%, *n*=8)	0.034

S. pneumoniae was most commonly isolated in central India. This variation is likely due to different patient populations and referral patterns in these two regions. In previous studies reported from the eastern and southern parts of India, *S. pneumoniae* was identified as the most prevalent bacteria by Lalitha *et al*.[9] and Das *et al*.,[10] whereas *S. aureus* was reported as the most prevalent bacteria by Kaliamurthy *et al*.[5] The finding of the present study indicates a shifting prevalence of bacterial isolates from the north to the central and southern parts of India.

In this study, although the susceptibly of *S. aureus* to vancomycin was the highest amongst all the antibiotics, there were vancomycin-resistant isolates. However, contrary to our findings, vancomycin-resistance was not reported in previous studies from the southern[9] or eastern parts of India.[10] This is a disturbing observation because vancomycin is still a sight-saving drug in more serious ocular conditions. No variation was reported in the susceptibility of *S. aureus* to vancomycin between north and central India. In our study, moderate susceptibility of *S. aureus* to aminoglycoside antibiotics (amikacin, tobramycin) and moderate (cefuroxime, cefazolin) to low (ceftazidime) susceptibility to cephalosporin was observed in both central and north zones. In contrast to our results, studies from United Kingdom reported high (87%–100%) susceptibility of gram-positive bacteria to cephalosporins.[2,11] Significant difference in *S. aureus*'s susceptibility to amikacin between north and central India was observed in our study. Isolates from north India were more susceptible to amikacin as compared to central India. Low susceptibility (<50%) of *S. aureus* was observed to second and fourth generation fluoroquinolones (ciprofloxacin, moxifloxacin, ofloxacin, gatifloxacin). Chawla *et al*.[12] had also reported resistance of ocular pathogens to fourth generation fluoroquinolones. The fourth-generation fluoroquinolones are being increasingly used as empirical therapy for bacterial keratitis.[13] Chawla *et al*.[12] suggested the use of fourth-generation fluoroquinolones as

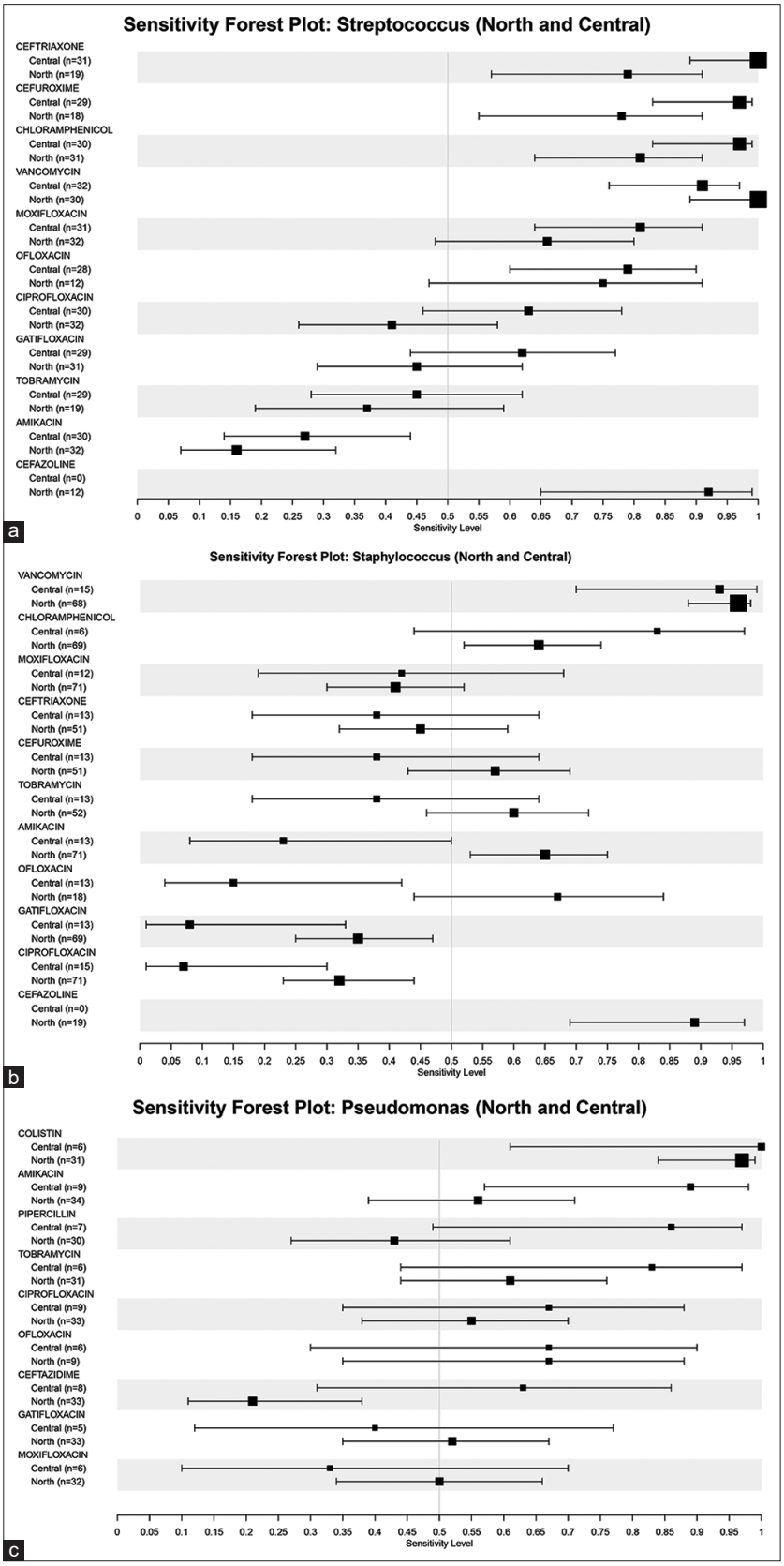

Figure 1: Forest plot comparing antibiotic sensitivity of bacterial isolates in north and central India for *Streptococcus pneumoniae* (a), *Staphyloccus aureus* (b) and *Pseudomonas aeruginosa* (c). The integers in x-axis represents the sensitivity level, the length of the horizontal lines represents sensitivity with 95% confidence interval of antibiotic sensitivity, and the box represents point estimate of antibiotic sensitivity of a particular antibiotic. The size each box is proportional to the number of antibiotic sensitivity test conducted

empirical therapy in cases of suspected bacterial keratitis in place of combination of fortified cefazolin and aminoglycosides. Lalitha *et al*.[9] and Das *et al*.[10] also reported similar results. Ting *et al*.[14] also identified a trend of moderate susceptibility of gram-positive bacteria to fluoroquinolones from studies reported from the United Kingdom. Ray *et al*.[15] reported that prior use of fluoroquinolones can be associated with antibiotic resistance. Interestingly, a significantly greater number of *S. aureus* isolated from north India were susceptible to ofloxacin as compared to those isolated from central India. Ofloxacin is still not a first line of choice of antibiotics in bacterial ulcers as it is a second generation fluoroquinolone.

In our study, more than 90% of the *S. pneumoniae* isolate showed susceptibility to vancomycin and β-lactam antibiotics. Similar susceptibility pattern of *S. pneumoniae* to vancomycin has been reported in previous studies from India,[9,10,12] and other countries.[16,17] Additionally, all isolates from central India were sensitive to ceftriaxone compared to 79% of isolates from north India. Among fluoroquinolones in both zones, *S. pneumoniae* isolates were moderately susceptible to ofloxacin, moxifloxacin, gatifloxacin, and ciprofloxacin. Chawla *et al*.[12] reported that *S. pneumoniae* exhibited resistance to fourth-generation fluoroquinolones. Kaye *et al*.[16] also reported that ciprofloxacin and ofloxacin were less active against *S. pneumoniae*. These findings are in contrast to that of Lalitha *et al*.[9] who reported high susceptibility of *S. pneumoniae* to ofloxacin and other fluoroquinolones. Low susceptibility of *S. pneumoniae* to aminoglycoside (amikacin, tobramycin) antibiotics was seen in our study in contrast to Chawla *et al*.[12] who reported 75%–100% susceptibility to Tobramycin.

The highest susceptibility of *P. aeruginosa* was reported to be to ofloxacin (66.6%). This finding aligns with an earlier study by Asbell *et al*.,[17] who reported 66.5% susceptibility of *P. aeruginosa* to ofloxacin. However, they reported high susceptibility of *P. aeruginosa* to chloramphenicol (94.3%).[17] They also reported no change in antibiotic susceptibility of *P. aeruginosa* over a 10-year period.[17] In contrast to this, our study revealed low susceptibility of *P. aeruginosa* to chloramphenicol (20.5%). Mun *et al*.[18] reported that *P. aeruginosa* were sensitive to ceftazidime in their patients. Das *et al*.[10] reported high susceptibility of *Pseudomonas* spp. to ciprofloxacin, ofloxacin, gatifloxacin, and moxifloxacin. A previous report from south India demonstrated that gatifloxacin was effective against the majority of gram-negative bacteria (~90%), including *P. aeruginosa*.[5] In another study from south India, Lalitha *et al*. reported that *P. aeruginosa* was susceptible to ofloxacin (86.9%), and had a similar susceptibility to other fluoroquinolones and aminoglycosides.[9] They also reported that the susceptibility pattern of *P. aeruginosa* was stable during the 10-year study duration.[9] However, in our study, *P. aeruginosa* did not show more than 90% susceptibility to any antibiotic tested, thereby highlighting the need to explore newer antibiotics as first-line drugs in *Pseudomonas* keratitis. The susceptibility of *P. aeruginosa* to ceftazidime was significantly higher in north India compared to central India in our study.

There are a few limitations to this study. The Kirby–Bauer disc diffusion method used by three participating institutes is not an automated test and does not give quantitative results or the minimum inhibitory concentrations of drugs. As this study was conducted at a tertiary eye care setting where patients with more severe disease were treated, the findings may not parallel the susceptibility pattern of bacteria in the community. All centers are referral centers so the cohort does not represent the true picture of antibiotic resistance. All cases must have already received a variety of antibiotics which could modify the sensitivity pattern. The one-year duration of the study period did not allow for analysis of temporal trend in the susceptibility patterns. As the study was conducted in multiple centers, there may be variations in the conduction of the tests or in interpretation of data. However, all these centers followed a uniform protocol which would have mitigated most of the variation. The multi-centric nature of the study is a novel approach to study the antibiotic susceptibility pattern over a wide area, which is a strength. This study provides key information about the current status of susceptibility patterns of identified bacterial isolates at large institutes in north and central India.

Conclusion

In conclusion, the prevalence of *S. aureus* and *P. aeruginosa* was higher in north and of *S. pneumoniae* in central India. There is a geographical difference in susceptibility pattern. *S. aureus* susceptibility to amikacin was significantly greater in north than in central India, whereas *S. pneumoniae* susceptibility to ceftriaxone and *P. aeruginosa* to ceftazidime was significantly greater in central India. The susceptibility of *P. aeruginosa* to different antibiotics was less as compared to other bacterial isolates, as well as to its previously reported susceptibility in studies from India and other countries. It is important to note that prevalence of bacteria and their susceptibility to antibiotics are not uniform across geography. We recommend hospitals should draft antibiotic policies based on their own culture findings, and culture of corneal scrapings should be a part of the treatment protocol for every case of infectious keratitis.

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Conflicts of interest

There are no conflicts of interest.

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Annexure-I

Laboratory procedures:

[A] Collection of specimens:

Corneal scrapings from both the leading edge as well as base of each ulcer were collected under aseptic conditions by an ophthalmologist under the magnification of a slit lamp beam after instillation of 0.5% proparacaine, using a Bard Parker 15 no. blade. The scrapings were processed as follows: First set of scraping was applied to two sterile slides for 10% Potassium hydroxide (KOH) mount preparation and Gram's stain procedure. Ziehl-Neelsen (ZN) 1% and 20% staining was done when required. Second set of scraping was inoculated onto solid media like blood agar and chocolate agar by 3 'C' streak method. Third set of scraping was inoculated onto Sabouraud dextrose agar (SDA) slants devoid of antibiotics and cycloheximide.

[B] Specimen processing:

In the ocular microbiology lab, the following tests were performed on the specimens that were collected.

(a) KOH wet mount preparation was done as following:

1. A clean glass slide was taken.
2. The specimen was placed in the center of the slide.
3. A drop of 10% KOH was added and a coverslip was placed over that and observed under microscope.

(b) Gram staining was done as following

1. Thin smear of the specimen was prepared on a clean sterile glass slide.
2. Then the smear was fixed by heating over a bunsen burner flame.
3. The smear was flooded with 1% gentian violet for 1 minute & washed with distilled water.
4. The smear was flooded with gram's iodine for 1 minute and washed with distilled water.
5. Decolorized with acetone, washed with distilled water and counter stained with dilute carbol fuschin for 30 seconds.

Gram positive or Gram negative organisms or yeast cells and hyphae were looked for in Gram's stain preparation.

(c) Ziehl -Neelsen staining /modified acid fast staining was done as following:

1. Thin smear of the specimen was prepared and dried in air.
2. The smear was fixed by heating over a Bunsen burner flame.
3. The smear was flooded with strong carbol fuschin stain for 5 minutes.
4. Washed with distilled water and flooded with 1% sulphuric acid for 3 minutes.
5. Washed with distilled water and counter stained with 3%methylene blue for 3 minutes.
6. Washed with distilled water, dried, and examined under oil immersion microscope.

Bacterial culture plates and the inoculated enrichment medium were incubated at 370C. After overnight incubation, bacterial culture was confirmed by growth on blood agar, chocolate agar and MacConkey agar followed by standard biochemical tests according to the clinical and laboratory standards institute (CLSI) guidelines Subculture from the enrichment broth was made onto blood agar and chocolate agar plates and incubated at 370C for 7 days. Inoculated SDA slants were incubated at 300C for up to 14 days.

(d) Interpretation of Bacterial culture

Bacterial culture plates were observed for growth at 24 hours, 48 hours and till 7th day. The growth on cultures media were considered significant if following criteria were met:

1. If same organism is observed on more than one solid media.
2. If there is confluent growth at the site of inoculation on one solid media
3. If growth of one media is consistent with direct microscopic findings after Gram's Stain and 1% and 20% ZN staining.
4. Growth on one solid and one liquid media.

[C] Sensitivity testing of bacterial isolates:

In vitro susceptibility testing was performed by Kirby-Bauer disc diffusion method. The interpretation was done using Clinical and Laboratory Standards Institute's serum standards. The antibacterial agents used were consistently tested for their efficacy against standard American Type Culture Collection (ATCC) bacteria (Staphylococcus aureus ATCC, Str. Pneumoniae ATCC, Haemophilus influenzae ATCC, Pseudomonas aeruginosa ATCC, and Escherichia coli ATCC) as a general quality control laboratory procedure.

Reference:

Performance Standards for Antimicrobial Susceptibility Testing, 32nd Edition, Available at: https://clsi.org/standards/products/microbiology/documents/m100/