Research Article

Bacterial Conjunctivitis: Microbiological Profile, Antimicrobial Susceptibility Patterns and Recommendations for Treatment

Naz Perween[*], *Dakshina Bisht*[**], *Prabhav Aggarwal*[***]

Abstract

Conjunctivitis is one of the most common ocular infections, with bacterial infections accounting for 50-70% of all conjunctivitis cases. We conducted a study to determine the bacteria causing conjunctivitis and their antimicrobial susceptibility patterns at a large hospital and medical college in North India. Conjunctival swab specimens were obtained from 91 conjunctivitis patients attending the Ophthalmology out-patient department and were processed by Gram's stain, culture and antimicrobial susceptibility testing. Of the 91 samples collected, 46 showed growth of bacterial isolates; yielding total of 49 isolates. Gram positive and Gram negative bacteria comprised 77.5% and 22.4% respectively; including *Staphylococcus aureus* (31%), *Staphylococcus epidermidis* (27%), *Streptococcus pneumoniae* (16%), *Streptococcus pyogenes* (4%), *Pseudomonas aeruginosa* (10%), *Klebsiella* (8%), *E. coli* (2%) and *Proteus spp.* (2%). Most bacteria were susceptible to the newer generation fluoroquinolones, particularly gatifloxacin, and hence may be used if the treatment is warranted. Amikacin can also be used if Gram negative organisms are suspected. However, the use of antibiotics must be minimized and choice should be made on the basis of microbiological report.

Keywords: Bacterial conjunctivitis, Antimicrobial susceptibility, *Staphylococcus*, India.

Introduction

Conjunctivitis is a common non-traumatic disease of the eye characterized by pain, conjunctival hyperemia and discharge; common etiological agents being viruses, bacteria, allergens or chemical irritants. In India, the exact prevalence and incidence of bacterial conjunctivitis is not clearly known; however, international data estimates the prevalence of conjunctivitis in the United States as 1.35% per year.[1]

Bacteria are known to be closely associated with the eye adnexa, while the inner parts remain sterile; breach in the surface protective mechanism can result in surface bacterial infections such as conjunctivitis, scleritis, keratitis, blepharitis, canaliculitis, dacryocystitis as well as deeper infections.[2] Bacterial infections may account for up to 50-70% of all conjunctivitis cases.[3] These include both Gram positive organisms (such as *Staphylococcus aureus, Staphylococcus epidermidis*, *Streptococcus pneumonia,* etc.*)* as well as Gram negative bacteria (such as *Escherichia coli, Klebsiella spp.,* etc).

A meta-analysis of cases of bacterial conjunctivitis by Sheikh and Hurwitz revealed that the topical antibiotic treatment is associated with significantly better rates of early clinical and microbiological remission when compared to placebo, and also suggested that this benefit maybe maintained for late (days 6 to 10) clinical remission.[4] Fluoroquinolones, due to their efficacy against both Gram positive and Gram negative bacteria, as well as their low toxicity, are commonly prescribed topical antibiotics in conjunctivitis.[5] However, due to the emergence of resistance to common fluoroquinolones, new generation fluoroquinolones have been introduced.[5] Despite rising antimicrobial resistance, microbiological report is rarely sought for prescribing topical antibiotics. The present study was designed to attempt to identify the etiological agents of bacterial conjunctivitis and their antimicrobial resistance patterns especially against newer generation fluoroquinolones; and to make recommendations that may assist clinicians in choosing appropriate empiric therapy.

[*]Assistant Professor/ cum-Junior Consultant, Superspeciality Pediatric Hospital & Post Graduate Teaching Institute, Sector-30, Noida.
[**]Professor & Head of the Department, Department of Microbiology, Santosh Medical College, Ghaziabad, India.
[***]Senior Resident, Department of Microbiology, Maulana Azad Medical College, New Delhi-110002.
Correspondence to: Dr Naz Perween, Assistant Professor/ cum-Junior Consultant, Superspeciality Pediatric Hospital & Post Graduate Teaching Institute, Sector-30, Noida. ***E-mail Id:*** drnaz07@gmail.com

Materials and Methods

The study was conducted at the Department of Microbiology of a large hospital and medical college in North India during 2011-12. During this period, patients presenting to the out-patient department (OPD) at the Department of Ophthalmology, with signs and symptoms of conjunctivitis were included in the study. Patients who had already received antimicrobial treatment were excluded. Written informed consent was taken from the patients/ guardians prior to inclusion in the study. Ethical clearance was taken from institutional ethical committee.

Detailed history was taken from patients regarding the predisposing factors; such as history of trauma to the eye, use of contact lenses, as well as medical and surgical history. For each patient, two sterile cotton swabs moistened with sterile physiological saline were swept along the inferior palpebral conjunctiva, avoiding contamination from the skin of the eyelid and the mucopurulent material accumulated at the inner canthus.

One swab was used to prepare smear on a clean glass slide, which was stained by Gram's staining and scanned under oil immersion lens. Second swab was inoculated on to nutrient agar, Mac Conkey's agar, blood agar and chocolate agar. All the culture plates were incubated at 37^{0}C and observed after 24 hours and 48 hrs of incubation. Identification of bacterial pathogens was based on colony morphology, staining characteristics and biochemical properties following standard laboratory protocol.[6]

Antimicrobial susceptibility testing was performed for all the bacterial isolates, as per the procedures described by CLSI (Clinical & Laboratory Standards Institute).[7] The choice of antibiotics tested was based upon common usage patterns and their availability as topical agent such as: chloramphenicol (30 µg), amikacin (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), moxifloxacin (5 µg) and gatifloxacin (5 µg). *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used for quality control.

Statistical Analysis

The data was presented as percentages and proportions. 95% confidence intervals were calculated wherever applicable. Fischer's exact test was used for testing the statistically significant differences. All calculations were done using SPSS version 20.

Results

Over a period of two years, a total of 91 patients met our inclusion and exclusion criteria and consented to be included in the study. Of the 91 cases, 49 (54%, 95% CI= 43.66%-63.73%) were male and 42 (46%, 95% CI= 36.27%-56.34%) were female. Age of the patients ranged from 14 to 70 years (median = 36 years). Majority of patients were between 20 and 40 years of age.

Gram's stained preparations showed Gram positive cocci in 24 (26%, 95% CI=18.36%-36.3%) cases (17 were arranged in clusters, while 3 were in chains, others were singularly/ randomly arranged) and Gram negative bacilli in 5 cases. Out of the total 91 cases, 46 (51%, 95% CI= 40.46%-60.59%) showed bacterial growth upon culture; no significant differences were found in cultures showing growths between males and females (p=0.2979). Cultures from 43 cases showed growth of single bacterial species, while three cases showed mixed bacterial growth; yielding a total of 49 bacterial isolates. Therefore, out of these 49 isolates, 43 (88%) grew as pure isolates, while six (12%) were obtained in mixed growth (Table 1). Gram positive cocci were more common and included *Staphylococcus aureus* (31%), *Staphylococcus epidermidis* (27%), *Streptococcus pneumoniae* (16%) and *Streptococcus pyogenes* (4%). Among Gram negative organisms, *Pseudomonas aeruginosa* (10%) was the most frequent organism followed by *Klebsiella spp.* (8%), *E. coli* (2%), and *Proteus spp.* (2%). Organisms obtained in mixed growth combinations are shown in Table 1.

Antibiotic Susceptibility Testing

The results of antimicrobial susceptibility testing are presented in Table 2. All the organisms showed better susceptibility to the newer fluoroquinolones (gatifloxacin and moxifloxacin) as compared to ciprofloxacin and ofloxacin. Preliminary direct Gram's staining may assist in deciding the empirical topical antimicrobial treatment, as shown in Table 3. There were no significant differences in the susceptibility profile of Gram positive and Gram negative organisms. Based on this, newer fluoroquinolones and aminoglycosides showed better activity against both Gram positive and Gram negative organisms, and may be recommended as drug of choice for empiric treatment. Multi-drug resistance (resistance to three or more drug classes) was seen in 17 (45%, 95% CI=30.14%-60.30%) and 6 (54%, 95% CI=27.99%-78.75%).

 ISSN: 0019-5138

Table 1.Bacterial Pathogens isolated from Conjunctivitis cases

Microorganisms	Number of Pure isolates	Number of isolates mixed with others	Total isolates (%)
Staphylococcus aureus	13	2	15 (31)
Staphylococcus epidermidis	12	1	13 (27)
Streptococcus pneumoniae	8	-	8 (16)
Streptococcus pyogenes	2	-	2 (4)
Pseudomonas aeruginosa	3	2	5 (10)
Klebsiella spp.	3	1	4 (8)
E.coli	1	-	1 (2)
Proteus spp	1	-	1 (2)
Total no. of isolates	43	6	49

Note: Figures in parentheses indicate percentages.

Table 2.Percentage of Strains susceptible to the Antimicrobial Agents tested

	Chloramphenicol	Amikacin	Gentamicin	Ciprofloxacin	Ofloxacin	Moxifloxacin	Gatifloxacin
Staphylococcus aureus (n=15)	67	74	87	69	73	87	87
Staphylococcus epidermidis (n=13)	69	92	85	53	62	85	92
Streptococcus pneumoniae (n=8)	100	68	75	88	75	100	100
Streptococcus pyogenes (n=4)	100	50	50	50	50	100	100
Pseudomonas aeruginosa (n=5)	80	100	80	60	80	100	80
Klebsiella spp. (n=4)	50	75	75	75	100	100	100
E.coli (n=1) *Proteus spp.* (n=1)	0 0	100 100	100 0	0 100	100 100	100 100	100 100

Table 3.Gram Positive, Gram Negative and Total Isolates Resistant to the Antimicrobial Agents Tested

	Resistant Gram positive isolates (n=38)	Resistant Gram negative isolates (n=11)	P value	Total isolates (n=49)
Chloramphenicol	9 (24)	5 (45)	0.2544	14 (29)
Amikacin	9 (24)	1 (9)	0.4193	10 (20)
Gentamicin	7 (18)	3 (27)	0.6727	10 (20)
Ciprofloxacin	14 (37)	4 (36)	1.0	18 (37)
Ofloxacin	12 (31)	1 (9)	0.2461	13 (27)
Moxifloxacin	4 (11)	1 (9)	1.0	5 (10)
Gatifloxacin	3 (8)	1 (9)	1.0	4 (8)

Note: Figures in parentheses indicate percentages.

Discussion

Ninety-one patients were included in the study, with number of male patients (54%) almost equal to that of female patients (46%); most patients were in the prime of their life (20-40 years of age). This finding is similar to those of a ten years long study by Cavuoto et al. in 2007.[8]

Direct Gram's staining of the conjunctival swab specimen may assist in initiating the appropriate empirical therapy; though not all cases of culture positive conjunctivitis showed bacterial elements on

direct Gram's staining. Previous studies have shown that bacterial ocular infections are more frequently caused by Gram positive bacteria; and in case of conjunctivitis, they have been reported to be responsible for as many as 90.9% of the cases[9]. In our study, 49 bacterial isolates were cultured from the conjunctival swabs taken from the patients; Gram positive cocci being much more common as compared to the Gram negative bacteria (78% vs. 22%). Abdullah FE et al. have reported *Staphylococcus aureus* as the most common cause of bacterial conjunctivitis, as was in our study.[9] Similarly, in another study from United States, *Staphylococcus aureus* was the most common organism among the bacterial conjunctivitis isolates.[10] *Staphylococcus epidermidis* isolated in our study as well as in other studies may represent an opportunist pathogen or simply a commensal; though distinction is difficult.[9]

In the present study, *Pseudomonas aeruginosa*, *Klebsiella spp.*, *E. coli* and *Proteus spp.* were the Gram negative bacteria isolated. Another study from New Delhi, India has also reported *Pseudomonas, Klebsiella* and *E. coli* as the most common Gram negative bacteria.[11]

Choice of antimicrobial drugs for conjunctivitis is limited by their availability for topical application. Sharma S has enlisted the drugs available for this purpose: fluoroquinolones, aminoglycosides, penicillins, chloramphenicol, tetracyclines and erythromycin/ azithromycin.[2] Fluoroquinolones are the most common topical antibiotics used in bacterial conjunctivitis,[5] but there are studies suggesting development of resistance against second generation fluoroquinolones like ciprofloxacin and ofloxacin[5,8]; therefore, the newer generation fluoroquinolones such as moxifloxacin and gatifloxacin were also tested for, along with ciprofloxacin and ofloxacin.

In our study, an overall large number of bacterial isolates were found to be susceptible to gatifloxacin (96%). Similarly, Bharathi et al. (2010) has also reported high susceptibility to gatifloxacin (93.68%).[12] We also found that resistance to gatifloxacin and moxifloxacin was lower as compared to ofloxacin and ciprofloxacin. Similar observations were made by Reddy et al., where susceptibility to gatifloxacin was the highest (85.6%) followed by ofloxacin (65.6%), moxifloxacin (63.9%) and ciprofloxacin (60.5%).[13]

Whereas, we have found gatifloxacin and moxifloxacin to be almost equally effective against both Gram positive and Gram negative bacteria, Bharathi et al. have reported Gram positive cocci to be more sensitive to moxifloxacin and vancomycin, and Gram negative isolates to be more sensitive to amikacin and gatifloxacin.[12] Ramesh S et al. concluded that cefazolin, vancomycin and chloramphenicol demonstrated the greatest efficacy against Gram positive isolates alone, while gatifloxacin and ofloxacin demonstrated the greatest efficacy against both Gram ositive and Gram negative[14]; however, as per our study, ofloxacin appears to have low efficacy against Gram positive.

Aminoglycosides are considered to be more effective against Gram negative organisms. In our study, we tested two aminoglycosides, amikacin and gentamicin; although we found Gram negative isolates to be least resistant to amikacin (9%), the resistance to gentamicin was 27%; this is not surprising in Indian scenario as gentamicin is widely used especially for topical use in eye infections.

Topical antibiotics are commonly prescribed in all cases of red eye; with the possibility of inappropriate treatment of viral conjunctivitis. This raises concerns of antibiotic resistance, costs and complications due to ocular or systemic antibiotic use. Moreover, use of topical antibiotics in all cases of red eye may result in delayed diagnosis of other non-infective conditions resembling conjunctivitis, such as iritis and acute angle closure glaucoma. Visscher KL et al. suggest that even if antibiotics are being prescribed to cover the bacterial causes, it becomes essential to consider whether antibiotics are even necessary for the resolution of bacterial conjunctivitis. They have also suggested that antibiotics may be prescribed after a delayed period, if symptoms do not improve within 3 days of onset, or not at all.[15]

In view of this and also of the rising resistance among both Gram positive and Gram negative bacteria, we recommend that microbiological testing of conjunctival swabs, especially in non-resolving cases. The results of microbiological culture are usually available in 2-3 days and should serve as a guide to begin use of topical antibiotics; in more severe cases, Gram's staining may be used to guide treatment as results can be made available immediately.

Thus to conclude, Gram positive organisms such as *Staphylococcus aureus* and *Staphylococcus epidermidis* are the common bacterial agents causing conjunctivitis. Since most bacteria are most susceptible to the newer generation fluoroquinolones, particularly gatifloxacin, these appear to be a suitable choice, if use of antibiotics is considered appropriate. Use of amikacin is also recommended particularly if Gram negative organisms are suspected. It should always be remembered that use of topical solutions containing a cocktail of antibiotics

and anti-fungals may provide immediate and short-term benefit, but is likely to limit availability of effective antibiotics in the long-term.

Source of Funding: Departmental funding

Conflict of Interest: None

References

1. Smith AF, Waycaster C. Estimate of the direct and indirect annual cost of bacterial Conjunctivitis in the United States. *BMC Ophthalmol* 2009; 9: 13.
2. Sharma S. Antibiotic resistance in ocular bacterial pathogens. *Indian J Med Microbiol* 2011; 29(3): 218-22.
3. Rietveld RP, ter Riet G, Bindels PJ, Sloos JH, van Weert HC. Predicting bacterial cause in infectious conjunctivitis: Cohort study on informativeness of combinations of signs and symptoms. *BMJ* Jul 2004; 329(7459): 206-10.
4. Sheikh A, Hurwitz B. Topical antibiotics for acute bacterial conjunctivitis: a systematic review. *Br J Gen Pract* 2001; 51: 473-77.
5. Cervantes LJ, Mah FS. Clinical use of gatifloxacin ophthalmic solution for treatment of bacterial conjunctivitis. *Clin Ophthalmol* 2011; 5: 495-502.
6. Forbes BA, Sahm DF, Weissfeld AS (Eds.). Bailey & Scott's Diagnostic Microbiology. 12th Edn. Missouri: *Elsevier*, 2007.
7. CLSI. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-first Informational supplement. CLSI document M100-S21. Wayne PA. Clinical and Laboratory Standards Institute, 2011.
8. Cavuoto K, Zutshi D, Karp CL, Miller D, Feuer W. Update on bacterial conjunctivitis in South Florida. *Ophthalmology* Jan 2008; 115(1): 51-56.
9. Abdullah FE, Khan MI, Waheed S. Current pattern of antibiotic resistance of clinical isolates among conjunctival swabs. *Pak J Med Sci* Jan-Mar 2013; 29(1): 81-84.
10. Adebayo A, Parikh JG, McCormick SA, Shah MK, Huerto RS, Yu G et al. Shifting trends in in vitro antibiotic susceptibilities for common bacterial conjunctival isolates in the last decade at the New York Eye and Ear Infirmary. *Graefes Arch Clin Exp Ophthalmol* Jan 2011; 249(1): 111-19.
11. Malhotra S, Mehta DK, Kumar P. Spectrum and antibiotic susceptibility pattern of bacterial isolates from conjunctival swabs. *Indian J Pathol Microbiol* Oct 2005; 48(4): 538-41.
12. Bharathi MJ, Ramakrishnan R, Shivakumar C, Meenakshi R, Lionalraj D. Etiology and antibacterial susceptibility pattern of community-acquired bacterial ocular infections in a tertiary eye care hospital in south India. *Indian J Ophthalmol* Nov-Dec 2010; 58(6): 497-507.
13. Reddy AK, Garg P, Alam MR, Gopinathan U, Sharma S, Krishnaiah S. Comparison of in vitro susceptibilities of Gram-positive cocci isolated from ocular infections against the second and fourth generation quinolones at a tertiary eye care centre in South India. *Eye (Lond)* Jan 2010; 24(1): 170-74.
14. Ramesh S, Ramakrishnan R, Bharathi MJ, Amuthan M, Viswanathan S. Prevalence of bacterial pathogens causing ocular infections in South India. *Indian J Pathol Microbiol* Apr-Jun 2010; 53(2): 281-86.
15. Visscher KL, Hutnik CML, Thomas M. Evidence-based treatment of acute infective conjunctivitis-Breaking the cycle of antibiotic prescribing. *Can Fam Physician* 2009; 55(11): 1071-75.