

## Bacterial Aetiology of Acute Infectious Conjunctivitis and their Antimicrobial Susceptibility Pattern

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### Abstract

**Introduction:** Pathogenic microorganisms cause ocular disease and the most frequently affected parts of the eye are the conjunctiva, lid and cornea. Conjunctivitis is the most common cause of “red eye”. The major bacterial causes of conjunctivitis are *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Klebsiella spp*, *Haemophilus influenzae*. It was evident that the irrational use of antibiotics has greatly increased the antibiotic resistance of eye-infecting bacteria and is turning the issue into a serious challenge for the fight against bacterial infections. **Objectives:** To isolate and identify the bacterial pathogens in acute infectious conjunctivitis and to observe their antimicrobial susceptibility pattern. **Materials and Methods:** This cross sectional study was carried out in the Department of Community Ophthalmology and Department of Microbiology & Immunology, Bangladesh Medical University (BMU) during March, 2021 to August, 2022. A total of 75 patients with the age of 10 years and above diagnosed clinically as a case of acute bacterial conjunctivitis were included in this study. All isolated organisms were tested for their in vitro antimicrobial susceptibility against various antibiotics using the Kirby-Bauer disk diffusion method. All the information was recorded in data collection sheets and analysed using SPSS and Microsoft Excel. **Results:** In this study, 26.7% cases had culture positive and 73.3% culture negative. *Staphylococcus aureus* (30.0%) was the most common organism. The isolates showed the maximum antibiotic sensitivity to moxifloxacin (75.0%) and gentamicin (75.0%) and the lowest to azithromycin (25.0%). **Conclusion:** Accurate diagnosis of the type of infection and its etiologic factors and prescription of suitable antibiotics may shorten the duration of the disease as well as transmission time.

**Keywords:** Acute bacterial conjunctivitis, Antibiotic resistance, Bacteria, Clinical feature, Conjunctival swab, Culture and Sensitivity.

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### Introduction:

Conjunctivitis is a term broadly used to describe an inflammation of the conjunctiva. Due to its common occurrence, contagiousness, and potentially debilitating morbidities, conjunctivitis is a global health care and economic burden. It is a common condition of the eye that occurs worldwide and affects all ages and social strata, affecting more than 2 percent of the population. It is caused by a variety of bacterial or viral pathogens but may also be caused by allergies, irritants or medications<sup>1</sup>. Bangladesh has seen a recent surge of infectious conjunctivitis nationwide and reported incidence is now 33.33% in some eye outpatient departments (OPDs) of the country, which is surprisingly higher than the usual expected rate of the disease<sup>2</sup>. Conjunctiva is a thin, translucent membrane lining the anterior part of the sclera and inside of the eyelids. It has 2 parts, bulbar and palpebral. The bulbar portion begins at the edge of the cornea and covers the visible part of the sclera; the palpebral part lines the inside of the eyelids. Inflammation or infection of the conjunctiva is known as conjunctivitis and is characterized by dilatation of the conjunctival vessels, resulting in hyperemia and edema of the conjunctiva, typically with associated discharge<sup>3</sup>. Conjunctivitis may be split into four main aspects; bacterial, viral, allergic and irritant. Both gram-negative (*H. influenza*, *H. aegyptius*, *Klebsiella pneumoniae*, and *Moraxella* spp.) and gram-positive (*S. aureus* and *S. pneumoniae*) bacteria were common pathogen for bacterial conjunctivitis. Patients with infectious conjunctivitis secondary to bacterial etiologies may benefit from antibiotics or close observation. However, from an antibiotic stewardship standpoint, there is a concern that mass antibiotic distribution may facilitate the spread of antibiotic resistance<sup>4</sup>. Bacterial conjunctivitis can be broadly split into three major categories; hyperacute bacterial conjunctivitis, acute conjunctivitis and chronic conjunctivitis. Hyperacute bacterial conjunctivitis is commonly seen in patients affected with *Neisseria gonorrhoeae*. The onset is often rapid with an exaggerated form of conjunctival injection, chemosis and copious purulent discharge. Prompt treatment is essential to prevent complications. Acute bacterial conjunctivitis is the most commonly seen bacterial conjunctivitis and often presents with a typical presentation, time course and prognosis<sup>3,5</sup>. In a study done by Weiss et al, the most common pathogens in acute bacterial conjunctivitis were *Staphylococcus aureus*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Moraxella catarrhalis*, whereas in an older study done by Gigilotti et al, *Chlamydia trachomatis* was also commonly found in infected patients<sup>6,7</sup>. Bacterial conjunctivitis is a relatively common infection and affects all people, although a higher incidence is seen in infants, school children and the elderly. Although its incidence is continuing to decrease in developing nations, periodic rises in incidence are seen during the monsoon seasons in many countries such as Bangladesh, and thus, bacterial conjunctivitis is the most common cause of infective conjunctivitis in developing nations<sup>3</sup>. In certain bacterial conjunctivitis, it is essential to identify a pathogen. As mentioned, most causes of conjunctivitis are diagnosed and treated on a clinical exam basis, but in patients who are

particularly susceptible or immunodeficient patients, a microbiological diagnosis must be made to exclude harmful pathogens. The main features of acute bacterial conjunctivitis are swelling of the eyelids, significant conjunctival hyperemia, a large amount of purulent or purulent secretion, pseudomembrane and typical follicular formation, and subconjunctival hemorrhage<sup>3,5</sup>. The major bacterial causes of conjunctivitis are *Streptococcus pneumoniae* and *Haemophilus influenzae*<sup>8</sup>. In a study, *S. aureus* and *S. epidermidis* are the most common causes of conjunctivitis in all age groups, however, this condition decreases with age and is also influenced by other factors such as season and weather conditions. Of the 195 bacteria isolated, about 81.5% were *Staphylococcus epidermidis* and *Staphylococcus aureus* and the remaining 19.5% included other species. *Pseudomonas aeruginosa* was most resistant to ampicillin. In the case of *S. epidermidis* and *S. aureus*, the highest resistance was observed against erythromycin and the least resistance was against rifampicin and linezolid<sup>9</sup>. Microbial culture of 521 swab shows that 39.5% were deemed positive for bacterial culture. The isolation rate by bacteria species ranged from 0.5% to 28.2%. In *Staphylococcus aureus* isolates, ophthalmic antibiotic resistance varied from 10.5% for polymyxin B to 66.7% for erythromycin<sup>10</sup>. In a study frequency positive sample were 156/200(78%). Among the 156 positive samples 59.6 %were females, 41.0% in age group (< 15 years). Gram positive bacteria were the predominant isolates (73.1%). The major organism isolated was *S. aureus* (41%) and the least organism isolated was *Haemophilus* sp (2.6%). The maximum antibiotic sensitivity of the isolates was against gentamycin (94.20%) followed by chloramphenicol and ceftazidime (78.8%) while the maximum resistance was against tetracycline (94.2%) followed by amikacin (73.1%)<sup>11</sup>. Similar study showed that the 82% were Gram-positive, while 18% were Gram negative, with *Staphylococcus aureus* being the most prevalent bacteria (35%), closely followed by *Staphylococcus epidermidis* (32%). *Streptococcal pneumoniae* and *Enterobacter* spp had rates of 2% which were lower. The majority of organisms showed sensitivity to levofloxacin (43%), while there was only a small amount of resistance to polymyxin B (4%)<sup>12</sup>. The majority of cases with bacterial conjunctivitis are self-limiting; however, prompt antibiotic therapy speeds up recovery and decreases complications. Fluoroquinolones are frequently administered topical antibiotics for conjunctivitis due to their effectiveness against both gram positive and gram negative bacteria and also for their minimal side effects. However, new generation fluoroquinolones have been launched as a result of the development of resistance to traditional fluoroquinolones<sup>13</sup>. Moxifloxacin and gatifloxacin are now the best broad-spectrum antibiotics with aminoglycosides coming in second. However, a significant development in fluoroquinolone resistance in recent years necessitates cautious usage of these antibiotics. Vancomycin remains the most effective antibiotic for MRSA treatment<sup>14</sup>. Resistance to most antibiotic classes is developing, resulting in a decrease in the efficiency of several routinely used topical antibiotics. To stop or at least slow the current trend, a plan that includes

the prudent use of antibiotics as well as the development of new drugs with minimal resistance potential is necessary. Aim of the study was to isolate and identify the common bacteria causing acute bacterial conjunctivitis as well as to observe their antibiotic susceptibility which will help in the management of acute infectious conjunctivitis cases.

#### Materials and Methods:

This cross-sectional study was conducted in Department of Community Ophthalmology and Department of Microbiology & Immunology, Bangladesh Medical University (BMU), Dhaka, from March 2021 to August 2022. Total 75 patients with acute bacterial conjunctivitis were enrolled for study. Acute bacterial conjunctivitis was diagnosed by detailed history and clinical features like redness, purulent or mucopurulent discharge, watering, photophobia, foreign body sensation, pain, blurring of vision, congestion, chemosis and lid involvement, cornea involvement. Patients who were already under treatment with antibiotics, presenting with other causes of reeyes like uveitis, episcleritis, scleritis, acute keratitis, sub-conjunctival haemorrhage, acute glaucoma, patients with allergic conjunctivitis, phlyctenular conjunctivitis, contact lens related red eye and patients with history of ocular trauma were excluded from study. Purposive consecutive sampling technique was applied to collect the sample from the study population. Study procedure & microbial analysis: Specimen from conjunctival sac was collected aseptically for microbiological analysis & obtained by sterile cotton swabs from the conjunctival sac. The samples were transported to the laboratory immediately and processed for Gram staining and immediately inoculated into culture media like blood agar, chocolate agar, MacConkey, nutrient agar media. Gram staining was done to identify whether the organism grown was Gram-positive or Gram-negative. Gram positive bacteria were identified as dark purple colour & Gram negative bacteria as pale to dark red. Blood agar and chocolate agar was incubated at 37°C for 24 hours. The plates were observed after 24 hours and 48 hours for the presence of any growth. The isolated organisms were identified by using standard procedures. Colony characteristic were noted down. Antimicrobial Susceptibility Tests: The standardized Kirby-Bauer disc diffusion test of the Clinical and Laboratory Standards Institute (CLSI) was used for testing. The media used was Mueller-Hinton agar for non-fastidious organisms. 5% Sheep Blood agar was added to Mueller-Hinton agar for fastidious organism and it was read after 16-18 hours. Inoculum turbidity was adjusted to 0.5 McFarland turbidity tube. A lawn culture was made on the surface of medium using sterile cotton swabs and antimicrobial discs were applied. The plates were incubated for 18-24 hours for non fastidious and under 5% CO<sub>2</sub> for 24-48 hours at 37°C for fastidious organisms. The zones of inhibition were measured and reported as susceptible or resistant. For detection of Methicillin Resistant Staphylococcus aureus (MRSA), oxacillin disc (10 microgram) was used on Mueller-Hinton agar containing 2% sodium chloride. **Data collection:** The demographic information, relevant history, clinical examination findings and culture findings of all the study subjects were recorded in the data collection sheet. **Data Analysis:** The statistical analysis was carried out using the Statistical Package for

Social Sciences version 22.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Qualitative variables such as organisms cultured of this study have been expressed as frequency and percentage. Chi-square test was done to observe association between qualitative variables. The results were presented in tables, figures, diagrams and p value < 0.05 was considered significant.

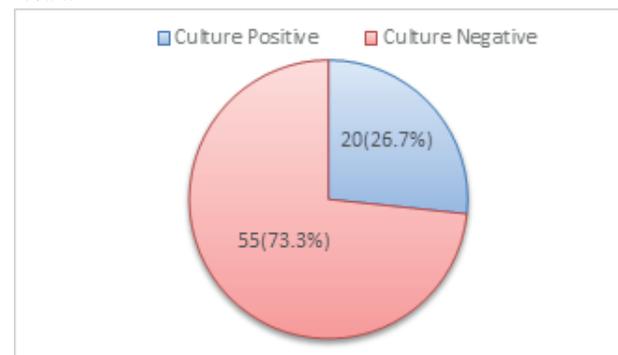
#### Result:

Table I showed demographic profile. It was observed that more than one third 26(34.7%) of subjects belonged to age 21-30 years. The mean age was 34.16±13.4 years. It was observed that almost three fourth 54(72.0%) subjects were male and 21(28.0%) were female.

**Table- I: Demographic profile of the respondents (n=75)**

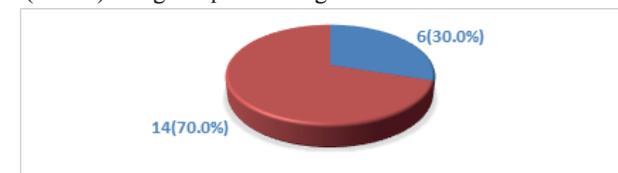
Variables	Frequency	Percentage
<b>Age (Yr.)</b>		
10-20	8	10.7
21-30	26	34.7
31-40	20	26.7
41-50	13	17.2
>50	8	10.7
Mean age (years)	34.16±13.4	
<b>Gender</b>		
Male	54	72.0
Female	21	28.0

Figure 1 shows the culture positivity in study population. It was observed that almost three fourth 55(73.3%) of subjects had culture negative and 20(26.7%) had culture positive result.



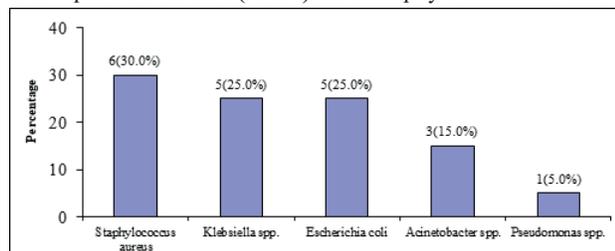
**Figure- I: Distribution of cases according to microbial culture finding (N=75)**

Figure 2 shows the gram positive and gram negative organisms in culture positive cases. It was observed that almost three fourth 14(70.0%) cases had gram negative and 6(30.0%) had gram positive organisms.



**Figure 2: Bar diagram shows gram positive and gram negative organisms in culture positive cases (N=20)**

Figure 3 shows the distribution of organisms in culture positive cases. It was observed that about one third 6(30.0%) subjects had *Staphylococcus aureus* followed by 5(25.0%) *Klebsiella* spp., 5(25.0%) *Escherichia coli*, 3(15.0%) *Acinetobacter* spp. and 1(5.0%) had *Pseudomonas* spp. All Gram-positive isolates (100%) were *Staphylococcus aureus*.



**Figure 3: Distribution of organisms in culture positive cases (N=20)**

Table II shows the drug sensitivity pattern of identified bacterial organisms. Moxifloxacin had sensitive in all 6(100%) cases of *Staphylococcus aureus*, all 3(100%) sensitive cases in *Acinetobacter* spp. and all 1(100%) sensitive case in *Pseudomonas* spp. Ciprofloxacin had 2(33%) sensitive and 4(67%) resistant cases in *Staphylococcus aureus*. Gentamicin had 4(67%) sensitive and 2(33%) resistant cases in *Staphylococcus aureus*, 4(80%) sensitive and 1(20%) resistant cases in *Klebsiella* spp. Chloramphenicol had sensitive in all 6(100%) cases of *Staphylococcus aureus*, 4(80%) sensitive and 1(20%) resistant cases in *Escherichia coli*, 2(67%) sensitive and 1(33%) resistant cases in *Acinetobacter* spp. and 1(100%) resistant case in *Pseudomonas* spp. Azithromycin had 2(33%) sensitive and 4(67%) resistant cases in *Staphylococcus aureus*, all 5(100%) resistant cases in *Klebsiella* spp., 2(40%) sensitive and 3(60%) resistant cases in *Escherichia coli*, all 3(100%) resistant cases in *Acinetobacter* spp. and 1(100%) sensitive case in *Pseudomonas* spp.

**Table II: Antibiotic sensitivity pattern of identified bacterial organisms (N=20)**

Drugs	Gram positive (n=6)		Gram Negative (n=14)			
	<i>Staphylococcus aureus</i> (n=6)		<i>Klebsiella</i> spp. (n=5)	<i>Escherichia coli</i> (n=5)	<i>Acinetobacter</i> spp. (n=3)	<i>Pseudomonas</i> spp. (n=1)
Moxifloxacin	Resistant	-	3(60%)	2(40%)	-	-
	Sensitive	6(100%)	2(40%)	3(60%)	3(100%)	1(100%)
Chloramphenicol	Resistant	-	3(60%)	1(20%)	1(33%)	1(100%)
	Sensitive	6(100%)	2(40%)	4(80%)	2(67%)	-
Tobramycin	Resistant	1(17%)	5(100%)	3(60%)	2(67%)	-
	Sensitive	5(83%)	-	2(40%)	1(33%)	1(100%)
Tetracycline	Resistant	1(17%)	3(60%)	2(40%)	3(100%)	1(100%)
	Sensitive	5(83%)	2(40%)	3(60%)	-	-
Gentamicin	Resistant	2(33%)	1(20%)	1(20%)	1(33%)	-
	Sensitive	4(67%)	4(80%)	4(80%)	2(67%)	1(100%)
Ciprofloxacin	Resistant	4(67%)	3(60%)	2(40%)	1(33%)	-
	Sensitive	2(33%)	2(40%)	3(60%)	2(67%)	1(100%)
Azithromycin	Resistant	4(67%)	5(100%)	3(60%)	3(100%)	-
	Sensitive	2(33%)	-	2(40%)	-	1(100%)

**Discussion:**

This cross sectional study was carried out with an aim to isolate and identify the common bacteria from the conjunctival swab of the patient with acute bacterial

conjunctivitis, to observe the antibiotic susceptibility pattern. The mean age of patients was 34.16±13.4 years. Similar study by Pandey et al. (2020) shows majority (e.g., 52) cases age belonged to 25-35 years<sup>15</sup>. Perween et al. (2016) where median age was 36 years and age of the patients ranged from 14 to 70 years<sup>16</sup>. In this current study it was observed that more than one fourth 26.7% subjects had culture positive and 73.3% had culture negative, which is similar with Al-Eryani et al. (2021), found that 39.5% were culture positive<sup>10</sup> and Abdullah et al. (2013) study showed that 41.0% were culture positive which were comparable with the present study<sup>17</sup>. Different interpretations of culture results may, at least in part, explain the variable incidence of positive bacteriology in various studies; some authors only provide the frequency of typically pathogenic bacteria, while others list all isolated bacteria. In this current study it was observed that nearly one third 30.0% had gram positive organisms and rest 70.0% had gram negative organisms. Mamota et al. (2019) found that majority 74.0% were gram positive bacteria followed by gram-negative bacteria 26.0%<sup>18</sup>. In this current study it was observed that about one third 30.0% subjects had *Staphylococcus aureus* followed by 25.0% *Klebsiella* spp., 25.0% *Escherichia coli*, 15.0% *Acinetobacter* spp. and 5.0% had *Pseudomonas* spp. Pandey et al. (2020) study showed that most common organism isolated coagulase positive *Staphylococci* 43.0% followed by *Klebsiella pneumoniae* 13.0%, *Pseudomonas* 6.0%, *Diphtheroids* 3.0%, *Alkaligenes fecalis* 2% which supports the present study<sup>15</sup>. Similar observations regarding the most common causative organism *Staphylococcus aureus* were also observed by Mamota et al. (2019)<sup>18</sup>, Perween et al. (2016)<sup>16</sup>, Abdullah et al. (2013)<sup>17</sup> and Adebayo et al. (2011)<sup>14</sup>. From the above obtained previous studies, it can be said that most common causative organism is *Staphylococcus aureus* in acute bacterial conjunctivitis which support with the present study. In this present study it was observed that 75.0% of cases had sensitive for moxifloxacin and gentamicin which were highest followed by 70.0% of cases had sensitive for chloramphenicol, 55.0% tetracycline, 50.0% ciprofloxacin, 45.0% tobramycin, 25.0% azithromycin whereas high level of resistance was seen azithromycin. Abdullah et al. (2013) study showed that overall antibiograms of bacterial isolates revealed that quinolones (sparfloxacin and moxifloxacin) and aminoglycosides (gentamicin and tobramycin) are the obvious drugs of choice for empirical therapy, followed by chloramphenicol which are comparable with the current study<sup>17</sup>. Previous study reported 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<sup>16</sup>. Similar observations regarding the low resistance to moxifloxacin were also observed by Adebayo et al. (2011)<sup>14</sup> and Cervantes and Mah, (2011)<sup>13</sup>. Abdullah et al. (2013) study mentioned that fourth-generation fluoroquinolone moxifloxacin inhibits both DNA gyrase and topoisomerase IV. This offers

improved effectiveness against both gram-positive and gram-negative organisms<sup>17</sup>. Ahmed and Hamdan, (2016) study found that gentamicin had the highest 94.20 % antibiotic sensitivity of the isolates, followed by chloramphenicol and ceftazidime at 78.8%<sup>11</sup> each which are comparable with present study. In this current study it was observed that Moxifloxacin and Chloramphenicol were 100.0% sensitive for *Staphylococcus aureus* followed by Tobramycin, Tetracycline, Gentamicin, Ciprofloxacin and Azithromycin. Furthermore, Ciprofloxacin and Azithromycin were 67.0% resistant for *Staphylococcus aureus*. Chloramphenicol was 100% resistant for *Pseudomonas* spp., Tetracycline was 100.0% resistant for *Acinetobacter* spp. and *Pseudomonas* spp., *Escherichia coli* and *Acinetobacter* spp. as well as Azithromycin was 100.0% resistant for *Klebsiella* spp. and *Acinetobacter* spp. Abdullah et al. (2013) study showed that moxifloxacin appears to be a superior broad-spectrum antibiotic with 86.3% sensitivity for gram positive isolates and 100.0% sensitivity for gram negative isolates which are comparable with present study<sup>17</sup>. Perween et al. (2016) study found that gatifloxacin and moxifloxacin are about equally efficient against gram positive and gram negative bacteria. Aminoglycosides are considered to be more effective against gram negative organisms which are comparable with present study. Moxifloxacin, gatifloxacin, and aminoglycosides showed the least amount of broad-spectrum antibiotic resistance<sup>16</sup>. In another study Mamota et al. (2019) showed that gentamicin is 100.0% sensitive to *pseudomonas* same as present study. The investigators found that gatifloxacin and moxifloxacin were most effective against gram-positive bacteria and ciprofloxacin is effective against gram-negative bacteria<sup>18</sup>. In all cases of red eyes, topical antibiotics are often recommended; viral conjunctivitis may be misdiagnosed and treated incorrectly. This raises issues about antibiotic resistance, costs and side effects from ocular or systemic antibiotic usage. Because of the increased resistance among gram positive and gram negative bacteria, this study recommends microbiological testing of conjunctival swabs, particularly in non-resolving cases.

#### Conclusions:

Present study concluded that *Staphylococcus aureus* was the most common organism responsible for acute bacterial conjunctivitis and the only bacteria found in gram positive cases. *Klebsiella* spp. & *Escherichia coli* were more frequent in gram negative bacteria. Moxifloxacin and gentamicin were the most sensitive to all isolates, whereas azithromycin was the least sensitive. Maximum sensitivity was observed to moxifloxacin and chloramphenicol in Gram-positive isolates and to gentamicin, moxifloxacin, chloramphenicol and ciprofloxacin in Gram-negative isolates. This work contributes to a better understanding of common organisms and antibiotic susceptibility, allowing ophthalmologists to manage acute bacterial conjunctivitis more effectively and reduce the risk of antibiotic resistance,

costs and its complication.

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