ACTA MEDICA SALINIANA

ORIGINAL PAPERS

MICROBIOLOGICAL EVALUATION OF BACTERIAL CONJUNCTIVITIS IN CHILDREN

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ISSN 0350-364X

DOI: 10.5457/600

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ABSTRACT

Background: Incidence and symptoms of bacterial conjunctivitis depend on etiological factors, clinical presentation, and age. The most common are Staphylococcus aureus, Haemophilus influenzae, Streptococcus pneumoniae, and Moraxella catarrhalis. Goals: To evaluate the most common microbial pathogens found in eye swabs and drug susceptibility of antimicrobial agents given in antibiogram.

Methods: Retrospective analysis of 73 microbiological findings (53 children) between January 2019 and March 2020 was conducted. The blood and chocolate Gram staining agar was used, incubated under 5-10% CO_2, 35-37˚C with daily reading. The following antibiotics were tested: chloramphenicol, ciprofloxacin, gentamicin, moxifloxacin, ofloxacin, trimethoprim/sulfamethoxazole, tetracycline, erythromycin, ampicillin, azithromycin, amoxicillin, clindamycin, and penicillin.

Results: In total, 53 children are included in this study, 43.39% male and 56.60% female, age ranged from 0-17 (mean age 2.7±4.4 years) mode of age was 2 years (75.5% cases). The number of performed eye swabs was 73, and pathogens were isolated in 95.9% of cases, and only in 4.1% cases, there were no pathogens in eye swabs. Among isolated pathogens, Staphylococcus aureus was proved to be the most common, in 27.4% of cases, the antibiotic with the highest drug susceptibility was chloramphenicol in 62.3% of cases.

Conclusion: Microbiological evaluation of bacterial conjunctivitis is reasonable in moderate to severe cases associated with mucopurulent discharge, prolonged treatment, and as confirmation of diagnosis. Choosing the accurate antibiotic therapy requires identification of the pathogen and assessing its susceptibility. Targeted treatment reduces the risk of antibiotic overdosing or unnecessary use of antibiotics. Prudent use of antibiotics reduces antimicrobial resistance.

Key words: conjunctivitis, children, microbiological evaluation, eye swab

INTRODUCTION

Conjunctivitis is an inflammation of the conjunctiva. It can be acute or chronic, with causative factors bacteria, viruses, and allergies and varying symptoms and clinical findings congruent with etiology. It is equally found in children and adults. Bacterial conjunctivitis is the most frequent with 80% of cases, viral in 13%, allergic in 2% cases, and idiopathic in 5% of cases. [1, 2] Conjunctivitis is the common reason for seeking professional aid, either by a primary care physician or an ophthalmologist. It is estimated that around 1% of patients in primary care or emergency rooms have conjunctivitis (70% of them having acute conjunctivitis). [3] Acute bacterial conjunctivitis is found in 1 out of 8 children, annually. Costs for conjunctivitis management are accordingly high with the estimated price of 589 million dollars in the USA. [2, 3]

Bacterial conjunctivitis is caused by environmental microorganisms, most commonly Staphylococcus aureus. [2] Other causative pathogens are Streptococcus pneumoniae and Haemophilus influenzae. [1, 2] Infection is spread with direct eye-hand contact, except in the case of neonates when the infection is transmitted via mother, and neonate conjunctivitis is caused by Neisseria gonorrheae and Chlamydia trachomatis. [3,4]

Diagnosis of acute conjunctivitis is clinical, followed by empirical treatment. [1-4] Symptoms and clinical findings are not always specific. The most common findings are conjunctival hyperemia, varying discharge (serous, seromucous, purulent, mucopurulent). Sometimes, membranes or pseudomembranes can be found, with

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Received:
7.2.2021.

Accepted:
3.11. 2022.

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Funding: none

Competing interests: none

or without hemorrhages. [1-4] A slit-lamp examination performed by a specialist is the only method that can accurately assess eye redness and discharge and make a differential diagnosis. [2]

Microbiological evaluation is not performed primarily and is indicated in cases of neonatal conjunctivitis, recurrent or refractory conjunctivitis [1, 3] as microbiological evaluation is expensive, and results are not readily available. These are the main reasons that eye swabs are not performed routinely. [3]

Conjunctivitis is a disease with complete resolution in the majority of cases. In some rare cases, there is the propagation of inflammation to the cornea leading to corneal ulcer formation. [4] Treatment consists of broad-spectrum topical antibiotic drops and ointment. Various antibiotics from different groups are used: aminoglycosides (gentamicin, tobramycin, neomycin, and framycetin), a group relatively cheap, but ineffective against S. pneumoniae. Second generation fluoroquinolones (ciprofloxacin, ofloxacin, and norfloxacin) are available, they are not expensive and have a good effect against H. influenzae, while third (levofloxacin) and fourth-generation (moxifloxacin, gatifloxacin, besifloxacin,) are fairly expensive, but they provide an effect on a wide spectrum of pathogens. [1-5].Topical macrolides (azithromycin, erythromycin) are well tolerable, available in an ointment form, less effective against H. influenzae, Streptococcus pneumoniae, and Staphylococcus aureus. [1- 5] Bacterial conjunctivitis treatment should avoid corticosteroid combination unless indicated, as corticosteroids can cause further deterioration in the cause of overlooked herpetic keratitis. [1]

METHODS

Retrospective evaluation and laboratory-based descriptive study of a total of 53 children between January 2019 to March 2020, with clinically diagnosed bacterial conjunctivitis in Private Ophthalmology Practice, were evaluated. In patients suspected of bacterial conjunctivitis an ophthalmological examination was performed, followed by an eye swab. 73 in total were then sent to the Microbiology Laboratory in the Private Policlinic Eurofarm. A complete ophthalmology examination of each eye including external examination of lids, conjunctiva, cornea with fluorescein dye test if needed were performed. Clinical changes like lid edema, conjunctival discharge, various lesions including papillae, follicles, hemorrhages, and membrane formation were documented. The cornea was examined for any evidence of marginal keratitis, infiltration, ulceration, vascularization, or similar.

Conjunctival swab protocol

The conjunctival swab was taken with a sterile cotton-tipped swab from the tarsal conjunctiva of the lower eyelid. The swab was then placed in a tube filled with sterile saline solution providing a delay in transport for a few hours without the risk of specimen desiccation. The collected specimen was kept at room temperature until the culture was made, using first the Gram staining method. The following bacterial culture methods used were: blood agar, selective chocolate agar for Haemophilus influenzae, and endo agar. The incubation period for blood agar in condition with 5-10% CO_2, temperature 35-37º is 40-48 hours with everyday reading, chocolate agar conditions: 5-10 % CO_2, temperature 35-37°, 40-48 hours with everyday reading. Antibiotic susceptibility testing was performed by dipping standard bacterial suspensions and plating on Mueller-Hinton agar sensitivity plates. Various antibiotic sensitivity discs (six per plate) were placed on each plate and incubated at 37°C for 24 hours. The bacterial pathogens were tested against the following antibiotics: chloramphenicol, ciprofloxacin, gentamicin, moxifloxacin, ofloxacin, trimethoprim/sulfometoxazole, tetracycline, erythromycin, ampicillin, azithromycin, amoxicillin, clindamycin, and penicillin. After 24 hours of incubation, the plates were examined for zones of inhibition around each of the antibiotic discs. Tables and graphs are used for result presentation. A descriptive and statistical study was done, using the number of cases, percentages, mean value, and standard deviation. For statistical analysis, MedCalc v12.3 (Antwerp, Belgium) statistical program for biomedical research was used.

RESULTS

We retrospectively evaluated 53patients, 23 males (43.39%) and 30 females (56.63%). (Table 1)

Table 1. Sex distribution in patients

Sex	No (100.00%)
Male	23 (43.39)
Female	30 (56.63)

Mean age of patients was 32.3 ±52.3 months or 2.7±4.4 years (Range of age: 13 days to 17 years). The majority of patients were two years old in 40 (75.5%) cases, the least number of patients were in the age group 5-10 years old, only in 3 (5.7%) cases. In age groups 2-5 years and 10-17 years there was an equal number of patients; 5 (9.4%) patients in each group. (Table 2)

Table 2. Age distribution of patients

Age	No (%)
0-2	40 (75.5)
2-5	5 (9.4)
5-10	3 (5.7)
10-17	5 (9.4)
Total	53 (100.00)

Right eye was affected in 15 (28.3%) cases, left eye in 18 (34.0%) cases, both eyes affected in 20 (37.73%) cases. (Table 3)

Table 3. Distribution and percentage of the affected eye

Affected eyes	Patients No (%)	Swabs No
Right eye	15 (28.3)	15
Left eye	18 (34.0)	18
Both eyes	20 (37.7)	40
Total	53 (100.00)	73

Normal flora was found in 3 (4.12%) cases, in those cases an antibiotic susceptibility testing was not performed. Pathogenic bacteria were seen in 70 (95.9%) cases. The most prevalent pathogen was Staphylococcus aureus in 20 (27.39%) cases, followed by Haemophilus influenza in 13 (17.8%), Streptococcus pneumoniae in 12 (16.43%), MRSA in 7 (9.58%), Moraxella catarrhalis in 5 (6.84%), Staphylococcus epidermidis in 3 (4.109%), Escherichia coli in 3 (4.12%), beta-haemolytic Streptococcus in 3 (4.12%), coagulase-negative Staphylococcus in 2 (2.74%), Enteroccocus species in 1 (1.36%), Klebsiella species in 1 (1.36%), Pseudomonas aeruginosa in 1 (1.36%), Enterobacter species in 1 (1.36%) and Proteus species in 1 (1.36%) case. Gram positive (Gram+) pathogen bacteria were found in 7 (50%) cases and Gram negative (Gram -) equally in seven (50%) cases.(Table 4)

Table 4. Distribution of detected pathogen in conjunctival swab

Type of pathogen bacteria	No (%)
Staphylococcus aureus Gram +	20 (27.39)
Haemophilus influenzae Gram -	13 (17.80)
Streptoccocus pneumoniae Gram+	12 (16.43)
MRSA Gram+	7 (9.58)
Moraxella catarrhalis Gram-	5 (6.84)
Staphylococcus epidermidis Gram+	3 (4.12)
Escherichia coli Gram-	3 (4.12)
Beta-haemolytic streptococcus Gram+	3 (4.12)
Coagulase-negative staphylococci Gram +	2 (2.74)
Enterococcus species Gram+	1 (1.36)
Klebsiella species Gram -	1 (1.36)
Pseudomonas aeruginosa Gram-	1 (1.36)
Enterobacter species Gram-	1 (1.36)
Proteus species Gram-	1 (1.36)
Total	73 (100.00)

The pathogens demonstrated relatively good susceptibility to chloramphenicol in 33(62.3%), followed by ciprofloxacin in 30 (41.5%), gentamicin in 19 (35.8%), moxifloxacin in 14 (26.4%), ofloxacin in 9 (16.9%), trimethoprim/sulfamethoxazole in 8 (15.1%), tetracycline in 7 (13.2%), erythromycin in 6 (11.3%), ampicillin in 4 (7.5%), azithromycin in 3 (5.7%), amoxicillin in 2 (3.8%), clindamycin in 2 (3.8%) and penicillin in 1 (1.9%) case. In 3 (4.1%) cases, Antibiotic susceptibility testing was not performed because normal flora (Staphylococcus epidermidis) was found. Drug susceptibility to 6 or more antibiotics was found in 1 (1.9%) case, to five antibiotics in 2 (3.8%) cases; to four antibiotics in 7 (13.2%) cases, to three antibiotics in 17 (32.1%) cases, to two antibiotics in 15 (28.3%) cases, to only one antibiotic in 8 (15.1%) cases. (Table 5)

Table 5. Estimated percentage of antimicrobial susceptibility

Antibiotics used in antimicrobial susceptibility test	No (%)
Chloramphenicol	33 (62.3)
Ciprofloxacin	30 (41.5)
Gentamicin	19 (35.8)
Moxifloxacin	14 (26.4)
Ofloxacin	9 (16.9)
Trimethoprim/ sulfamethoxazole	8 (15.1)
Tetracyclin	7 (13.2)
Erythromycin	6 (11.3)
Ampicillin	4 (7.5)
Azithromycin	3 (5.7)
Amoxicillin	2 (3.8)
Clindamycin	2 (3.8)
Penicillin	1 (1.9)

Out of 33 (62.3%) cases of drug susceptibility to chloramphenicol, largest susceptibility was found in Staphylococcus aureus in 12 (36.36%) cases, followed by Streptococcus pneumoniae in 6 (18.18%), Haemophilus influenzae in 5 (15.15%), Moraxella catarrhalis in 1 (3.03%), Klebsiella in 1 (3.03%), coagulase-negative staphylococci in 2 (6.06%) and beta-haemolytic streptococci in in 0 (0.0%) cases. Four Gram positive and three Gram negative bacteria were detected. (Table 6)

Table 6. Drug susceptibility of bacteria to various antibiotics

Drugs/Pathogen bacteria	Chloramphenicol N=33	Ciprofloxacin N=30	Gentamicin N=19	Moxifloxacin N=14	Ofloxacin N=9	Trimethoprim sulfamethoxazole N=8	Tetracycline N=7	Erythromycin N=6	Ampicillin N=4	Azithromycin N=3	Amoxicillin N=2	Clindamycin N=2	Penicillin N=1
S. aureus	12	9	7	4	2	1	0	3	0	0	0	2	0
H. influenzae	5	6	1	4	1	0	3	1	2	1	1	0	1
S. pneumonia	6	4	4	4	1	6	1	0	0	2	1	0	0
MRSA	4	1	2	1	1	1	3	0	0	0	0	0	0
Moraxella catarrhalis	1	2	1	1	2	0	0	0	0	0	0	0	0
Staphyloccus epidermidis	0	0	0	0	0	0	0	0	0	0	0	0	0
Escherichia coli	0	2	1	0	1	0	0	0	0	0	0	0	0
Beta-haemolytic streptoccocus	0	2	1	0	0	0	0	0	0	0	0	0	0
Coagulase-negative staphylococci	2	1	0	0	0	0	0	1	0	0	0	0	0
Enteroccocus species	1	1	0	0	0	0	0	1	1	0	0	0	0
Klebsiella species	1	0	1		1	0	0	0	0	0	0	0	0
Pseudomonas aeruginosa	0	1	1	0	0	0	0	0	0	0	0	0	0
Enterobacter species	0	0	0	0	0	0	0	0	0	0	0	0	0
Proteus spp.	1	1	0	0	0	0	0	0	1	0	0	0	0

Various symptoms were present: conjunctival hyperemia in 41 (77.4%) cases, while in 12 (22%) cases no redness was present. In 3 (5.7%) cases no discharge was present. In 50 (94.4%) cases there was discharge; mucopurulent in 30 (56.6%) cases and serous in 20 (37.7%) cases. Membranes and hemorrhages were present in 1 (1.9%) case, and no corneal complications were found (Table 7).

Table 7. Frequency of signs and symptoms

Clinical signs and symptoms	No (%)
Eye redness	Yes 41 (77.4)
	No 12 (22.6)
Total	53 (100.0)
Discharge	50 (94.4)
No discharge	3 (5.7)
Total	53 (100)
Membranes and hemorrhage	1 (1.9)
Superficial keratitis	0 (0.0)

DISCUSSION

We retrospectively evaluated 73 microbiological findings of eye swabs in 53 children between January 2019 and March 2020, which included 23 males and 30 females. The youngest patient was 13 days old and the oldest patient is 17 years. Normal flora was seen in three cases while pathogenic bacteria was seen in 70 (95.9%) cases. The most prevalent pathogen bacteria found was Staphylococcus aureus in 20 (27.39%) cases. These pathogens demonstrated relatively good susceptibilities to chloramphenicol in 33 (62.3%) cases.

Okesola and Salako presented 342 conjunctival swabs of 365 patients with clinically diagnosed 216 (59.2%) males and 149 (40.8%) females, between 3 months and 88 years of age. Bacterial pathogens were detected in the conjunctival samples of 342 (93.7%) patients, while 23 (6.3%) showed no growth activity. Staphylococcus aureus was identified in 256 (74.9%) cases, Pseudomonas aeruginosa in 22 (6.4%) , Escherichia coli in 11 (3.2%) , coagulase-negative staphylococci in 35 (10.2%), Klebsiella species in 7 (2.1%) , Streptococcus pneumonia in 5 (1.5%), Haemophilus influenzae in 4 (1.2%) and Proteus mirabilis and Neisseria gonorrhoeae in 1 (0.3%). In this study, 67% of bacteria were susceptible to ceftriaxone and 39.2% were susceptible to chloramphenicol. [6]

Veena presented the highest incidence of conjunctivitis in the younger age group within 0-20 years (The youngest patient is 4 days old and the oldest patient is 75 years), 63 males and 37 females. The normal flora was isolated in 4 % of the cases while pathogenic bacteria were isolated in 53%. coagulase-positive staphylococci was present in 38.75% and Klebsiella pneumonia in 11.25% cases, which emerged as the first and second most common causative bacteria of conjunctivitis. Ciprofloxacin proved as the most effective drug. Conjunctivitis usually affected both eyes, either simultaneously or one after the other. Complications like subconjunctival hemorrhages were seen in 6% of the cases, superficial keratitis was seen in 4%. All the cases presented with red eyes, 26% tearing eyes, while 48% had discharge (mucoid, mucopurulent, or purulent). [7]

Patel et al. evaluated the usefulness of clinical signs and symptoms to distinguish bacterial from viral conjunctivitis in 111 pediatric patients. The mean age of the patients was 33 months, 78% had positive bacterial cultures and non-typeable Haemophilus influenzae accounted for 82% of bacterial infections. A history of sticky eyelids, in combination with a physical finding of mucoid or purulent discharge, was attributable to bacterial infection in 95% of cases. [8]

Meltzer et al. observed a study of 368 patients with conjunctivitis who were between six months and 17 years of age and 88% had a positive bacterial culture. The most common causative organisms included H influenza 67.6 % and S pneumonia in 19.7%, S aureus in 6%, and other in 2.2%. [9]

Gross et al. studied evaluated 141 children, aged 12 years or younger with culture-proven bacterial conjunctivitis, 45% with H influenzae, 30% with S pneumonia, and 25% with another infection and compared ciprofloxacin with tobramycin drops. After seven days, the clinical cure rates of ciprofloxacin and tobramycin were 99% and 97%, respectively, and the bacteriological cure rates were 90% and 84%, respectively. [10]

Block et al. reported bacterial eye infection in 250 children with acute conjunctivitis, the mean age of the children was 24 months. Bacterial cultures were positive for H influenza in 42% of cases, S pneumonia in 30% of cases, and were negative in 32% of cases. Erythema was present in 53% of cases, and purulent discharge in 83%. As for in vitro activity, ciprofloxacin, ofloxacin, and tetracycline were the most active; and gentamicin, tobramycin, polymyxin B-trimethoprim, and polymyxin B-neomycin were intermediately active. Sulfamethoxazole possessed no activity against either pathogen. [11] Rose et al. observed 326 children aged 6 months to 12 years with the clinical diagnosis of infective conjunctivitis in primary care, 163 children received chloramphenicol and 163 received placebo eye drops, and double-blind trial to compare the effectiveness of chloramphenicol eye drops with placebo in children with infective conjunctivitis in primary care. All children were followed up for 6 weeks to identify relapse. The study concluded that most children presenting with acute infective conjunctivitis in primary care will get better by even without antibiotic treatment. [12]

CONCLUSION

Bacterial conjunctivitis is a fairly common ophthalmological disease in children. Treatment is mostly empirical and based on symptoms and clinical findings, using the available antibiotics. Microbiological evaluation of conjunctival swab is indicated in cases of failed or prolonged disease, or in cases of relapse. On the other hand, microbiological evaluation is justified given the great number of possible pathogens, their susceptibility, and reduction of uncontrolled use of antibiotics which tends to lead to the formation of antimicrobial resistance. In our study, microbiological evaluation was justified in 95.9% of cases, the most common isolated pathogen was Staphylococcus aureus, and the highest susceptibility was attributed to the chloramphenicol.

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