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Detection of Bacterial Causes of Conjunctivitis Among Neonates and Under five Children and Their Antibiotic Sensitivity Pattern

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Key words:
Conjunctivitis, bacterial cause, antibiotic sensitivity.

Abstract:

Objectives: *The present study was designed to find out the bacterial causes of conjunctivitis among neonates and under five children and their antibiotic sensitivity pattern.* ***Methodology:*** *A cross sectional study was done in the department of Microbiology, Dhaka Medical College from July 2012 to June 2013. Conjunctival swab samples were collected from neonates and children of clinically suspected conjunctivitis attending OPD of Dhaka Medical College Hospital and National Institute of Ophthalmology and Hospital.* ***Results:*** *Out of 206 cases, 145 (70.73%) were culture positives. Most common gram positive bacteria was Staphylococcas aureus and gram negative bacteria was Pseudomonas aeruginosa. In neonates, Chlamydia trachomatis were detected by PCR. Organisms show highest sensitivity to vancomycin, amikacin, and chloramphenicol. C****onclusion****: So, early diagnosis of bacterial causes and its antibiotic sensitivity pattern will help in proper management of childhood conjunctivitis.*

Introduction:

Conjunctivitis is an inflammation of the conjunctiva.[1] Conjunctivitis occurs in all age groups and is caused by various infectious agents (bacteria, viruses and fungi) and noninfectious agents such as chemical and allergic.[2] Neonatal conjunctivitis is often known as Ophthalmia neonatorum. It is defined as conjunctivitis occurring in a newborn during the first month of life.[3] Acute conjunctivitis is the most common ocular infection in childhood, usually affecting children younger than 6 years old with a peak incidence between 12 and 36 months.[4] The causative organism of this infection has been documented as bacterial in 54 to 73% of pediatric cases.[5] The commonly isolated bacteria in case of neonatal conjunctivitis are *Chlamydia trachomatis* and *Neisseria gonorrhoeae*.[6] *Staphylococcus aureus* is the other most frequent organism, followed by *Streptococcus pneumoniae, Viridians streptococci, Staphylococcus epidermidis, Escherichia coli, Klebsiella spp., Citrobacter, Proteus, Enterobacter,* and *Pseudomonas* species.[7,8] In case of children, the causative bacteria are *Staphylococus aureus*,

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Pseudomonas sp., *Klebsiella sp.*, *Streptococcus pneumoniae*, *Haemophyllus influenzae*, *Escherichia coli* and *Proteus mirabilis*.[9] Ophthalmia neonatorum is the major cause of blindness in low income countries.[10] Chronic bacterial conjunctivitis in children can produce blepharitis and meibomian gland inflammation.[11] So the study has been done to establish proper treatment and minimizing potential complications of disease.

Methods and Materials:

A Cross sectional study was done in department of Microbiology, Dhaka Medical College, Dhaka, during the period of July 2012 to June 2013. The study population was neonates and children from one month to five years. 206 samples were collected from neonates and children under five years with conjunctivitis who attended the outpatient department of Dhaka Medical College Hospital and National Institute of Ophthalmology and Hospital.

Specimen collection: Sample was collected from conjunctival swabs of both eyes. In case of neonates, three conjunctival swabs were taken. One swab for gram staining, one swab for culture and one swab for PCR. Two conjunctival swabs were taken from children. One swab for gram staining and one swab for culture.

Isolation and identification of bacteria: Microscopic examination of conjunctival swab was done by gram stain for identification of bacteria and associated pus cells. Culture of conjunctival swab was done In Blood agar, Chocolate agar, Mac Conkey agar and Modified Thayer Martin (MTM) agar media. The bacterial pathogens were identified by their colony characteristics, gram staining and various biochemical tests. Sensitivity pattern of the isolated organism were determined by modified Kirby-Bauer technique using Mueller-Hinton agar media following CLSI guideline 2011[12]. The agar plates were incubated at 37^0 C. for 24 hours. The antimicrobial agents used were penicillin (10 unit/disc), gentamycin (10µg/disc), tetracycline (30µg/disc), erythromycin (15µg/disc), ciprofloxacin (5µg/disc), vancomycin (30µg/disc), tobramycin (10µg/disc), chloramphenicol (30µ g/disc), oxacillin/cefoxitin (1µg/disk or 30 µg/disc), moxifloxacin (5 µg/disc). amikacin (30µg/ml), amoxiclav (amoxicillin 20µg/disk and clavulanic 10µg/disk) and ampicillin (10µg/disc).

Polymerase chain reaction:

Polymerase chain reaction was done to detect genes of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in conjunctival swab. Oligonucleotide primers (1st base, Singapore) were used for PCR amplification of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* DNA. The primers were,

NG Forward 5'GCT ACG CAT ACC CGC GTT GC 3, NG Reverse 5'CGA AGA CCT TCG AGC AGA C 3' for *N. gonorrhoeae.*

KL1 5'TCC GGA GCG AGT TAC GAA GA 3' and KL2 5'AAT CAA TGC CCG GGA TTG GT 3' for *C. trachomatis.*

For DNA extraction, the test tube containing conjunctival swab in 2 ml of phosphate buffer saline (PBS) was taken. Then the sample was thawed, vortexed to make a homogenous suspension and about one ml was taken into two separate eppendrof tubes. This tubes were centrifuged at 12000g for 10 minutes and after removing the supernatant by aspiration, one pellet was suspended in 100µl of lytic buffer with nonionic detergent tween 20 (0.45%) and protinase K (200µg/ml) for *N. gonorrhoeae* and another with tween 20 (0.05%) and Proteinase K (100µg/ml) for *Chlamydia trachomatis*. These were then incubated, heat block (DAIHA Scientific, Seoul, Korea) was given for 10 minutes. The sample was kept on ice for 5 minutes, then again centrifuged at 13000g for 10 minutes. A 25ìL lysate of target cellular material was amplified through 36 cycles (1 min denaturized at 94 °C, 45 seconds primer annealing at 62 °C and 1 min 30 second primer extension at 72 °C for *N. gonorrhoeae*. For *C. trachomatis*, 45 second annealing step was at 55^0 C). For each sample, a total 25 µl of mixture was prepared by mixing of 12.5 µl of mastermix, 1µl of forward primer, 1 µl of reverse primer, 3 µl of DNA template and 7.5ml nuclease free water (Promega Corporation, USA). The band of PCR product was showed by electrophoreses. We found a 241 bp band for *C. trachomatis* on 1% agarose gel which had undergone electrophoresis for 20 minutes at 100 volts. *N. gonorrhoeae* was not detected by PCR.

Result:

A total 206 clinically suspected bacterial conjunctivitis cases were studied. Among them, 60 (29.12%) were neonates and 146 (70.88%) were children under 5 years. Among neonatal conjunctivitis cases, 42 (70%) and among children, 103 (70.54%) were culture positive. Among the culture positive cases of neonates, 26 (62%) were male and 16 (38%) were female and among children, 58 (56.30%) were male and 45 (43.70%) were female (table-I).

Table-I. *Age and sex distribution of culture positive cases in neonates and children (n=145)*

Age group	Culture positive cases	Male n (%)	Female n (%)
Zero- 30 days	42	26 (62.00)	16 (38.00)
One month- 5 years	103	58 (56.30)	45 (43.70)
Total	145	84 (59.93)	61 (42.07)

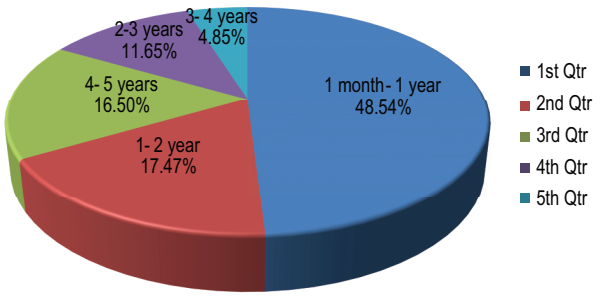

Fig- 1: *Age distribution of culture positive cases in children (n=103).*

Among the 145 culture positive cases of neonates and children under five years, the most predominant bacteria were *Staphylococcus aureus*. Followed by *Streptococcus pneumoniae, Staphylococcus epidermidis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia Coli, Diphtheroid spp, Viridans streptococci, Citrobacter freundii* and *Moraxella catarrhallis*. (Table-II).

Table-II. *Isolated bacteria from conjunctival swab samples in neonates and children by Culture (n= 145).*

Isolated bacteria	No. of isolates n (%)
Staph. aureus	47 (32%)
Strep. pneumoniae	29 (20%)
Staph. epidermidis	21(14%)
Pseudomonas aeruginosa	14 (10%)
Klebsiella pneumoniae	12 (8%)
Esch. coli	8 (6%)
Diphtheroid spp	6 (4%)
Viridans strepococci	4 (3%)
Citrobacter freundii	3 (2%)
Moraxella catarrhallis	2 (1%)
Total	145 (100)

Among the 60 suspected cases of neonates, 3 (5%) *Chlamydia trachomatis* were detected by PCR. (Table-III).

Table-III. *Detection of bacterial spp. from conjunctival swabs of neonates by PCR (n=60)*

Bacterial species	PCR positive n (%)	PCR negative n (%)	Total n (%)
C. trachomatis	3(5.00)	57(95.00)	60(100.00)
N. gonorrhoeae	0(0.00)	60(100.00)	60(100.00)

Table-IV shows the sensitivity pattern of gram positive bacteria to different antimicrobial agents. *Staphylococcus aureus* shows highest sensitivity to vancomycin (95.74%) and lowest sensitivity to tetracycline (53.2%). *Streptococcus pneumoniae* shows highest sensitivity to vancomycin, oxacillin (100%) and lowest sensitivity to tetracycline (37.93%). *Staphylococcus epidermidis* shows highest sensitivity to vancomycin, chloramphenicol (100%) and lowest sensitivity to penicillin (47.61%).

Table-IV. Antibiotic sensitivity pattern of gram positive bacteria

Antibiotic disk	*S. aureus*	*S. pneumoniae*	*S. epidermidis*
penicillin	27(57.45%)	27(93.10%)	10 (47.61%)
gentamycin	35(75.46%)	12(41.37%)	17(81%)
vancomycin	45(95.74%)	29(100%)	21(100%)
tetracycline	25(53.2%)	11(37.93)	11(52.38%)
erythromycin	27(57.45%)	21(72.4%)	12(57.14%)
moxifloxacin	43(91.48%)	25(86.20%)	19(90.43%)
oxacilin/ cefoxitime	38(80.86%)	29(100%)	18(85.71%)
chloramphenicol	40(85.1%)	27(93.1%)	21(100%)

Table-V. Antibiotic sensitivity pattern of gram negative bacteria

Antibiotic disk	*P. aeruginosa*	*K. pneumoniae*	*E. coli*
gentamycin	5(35.71%)	9(75.00%)	6(75.00%)
ciprofloxacin	12(85.71%)	7(58.33%)	3(37.50%)
amikacin	14(100%)	10 (83.33%)	8 (100%)
ceftazidime	10 (78.57%)	10 (83.33%)	6 (75.00%)
tobramycin	5 (35.71%)	7(58.33%)	6(75.00%)
chloramphenicol	10 (78.57%)	11(91.67%)	5(62.50%)

Table-V shows the sensitivity pattern of gram negative bacteria to different antimicrobial agents. *Pseudomonas aeruginosa* shows highest sensitivity to amikacin (100%) and lowest sensitivity to gentamycin and tobramycin (35.71%). *Klebsiella pneumoniae* shows highest sensitivity to chloramphenicol (91.67%) and lowest sensitivity to ciprofloxacin and tobramycin (58.33%). *Escherichia coli* show highest sensitivity to amikacin (100%) and lowest sensitivity to ciprofloxacin (37.50%).

Discussion:

Conjunctivitis is a common cause of paediatric primary eye care visits and a common ophthalmologic complain in the paediatric emergency department.[13] In the present study, among the neonatal conjunctivitis cases, 42 (70 %) were culture positive and among the suspected conjunctivitis cases in children, 103 (70.54%) were culture positive and the mean age was 36 months. The highest prevalence of conjunctivitis was in one to 12 months (48.54%). Pandey *et al.* reported 66.5% culture positive conjunctivitis cases in neonates. Patel *et al* reported 78% culture positive cases in children with the mean age of 33.2 months and Remco *et al.* reported that the incidence of conjunctivitis was maximum in children up to one year. In this study, *Staphylococcus aureus* (32%) including MRSA (19.14%) and VRSA (4.25%) were the most predominant bacteria among neonates and children. It was similar with the findings of Verma *et al* and Amini *et al.* In contrast, Khoshedi *et al.* isolated 14.2% and Samuel *et al.* reported 5.8% *Staphylococcus aureus* in their studies. The predominance of *Staphylococcus aureus* in our study may suggest that most of the cases of neonatal conjunctivitis were postnatally acquired rather than during passage through the birth canal. Virulence factors possessed by *S. aureus* make it to be the commonest agent of infection in children including neonates.[19] *Pseudomonas aeruginosa* (10%) was predominant gram negative bacteria in this study. Adeyeba *et al.* isolated 11.5% and Idu and Odjimogho reported 14% *Pseudomonas aeruginosa* in their study. In the present study, 3 (5%) *Chlamydia trachomatis* were detected by PCR in neonates. Amini *et al.* detected 2% and Yip *et al.* detected 12.5% *Chlamydia trachomatis* by PCR in their study. In contrast, Afjeiee *et al.* detected

16.6 % and Ingrid reported 64% *Chlamydia trachomatis* by PCR. The differences in detection of *Chlamydia* from different centres may be a reflection of the socioeconomic status, personal hygiene of the individuals and predominant agents in the newborn environment which may differ. It may also be due to variation in the aetiological agents of STIs and maternal genital flora [24]. In this present study, no *Neisseria gonorrhoeae* was detected by gram stain, culture and PCR. Similiarly, Verma *et al.* and Soltanzadeh *et al.* had not identified any *gonococcal* ophthalmia neonatorum in their studies. In contrast to the findings of the present study, Amini *et al.* isolated 3%, Afjeiee *et al.* isolated 3.7% and Abdulsalam *et al.* isolated 1.7% *Neisseria gonorrhoeae* in their studies. The low rate of *Neisseria gonorrhoea* isolation may be due to availability of health facilities, improved health habits, antenatal care attendance, awareness and actual improvement in managing cases of Gonorrhoea. In this present study, 98.58% gram positive cocci were sensitive to vancomycin and 94.44% gram negative bacilli were sensitive to amikacin. In case of both gram positive and gram negative bacteria 85.15% were sensitive to chloramphenicol. Different studies have shown that most isolated bacteria were sensitive to chloramphenicol[25,26,27] and the clinical cure rate by moxifloxacin was 91.1%[29]. Reduced sensitivity was found to gentamycin, tetracycline, erythromycin, ciprofloxacin and MRSA showed high rate of resistance to tetracycline, tobramycin and penicillin in the present study. Chalita *et al.,* and Block *et al.* found that *Streptococcus pneumoniae* and methicillin-resistant *Staphylococcus aureus* (MRSA) had exhibited high rates of resistance to tobramycin and gentamicin. Threefold increase in resistance to ciprofloxacin to gram positive bacteria was reported in a study by Cavuoto. Newer fluroquinolones were more active than the older ones against bacteria associated with conjunctivitis [31] So, early detection of causative bacteria and their antibiotic sensitivity pattern will help to reduce the complications of neonatal and childhood conjunctivitis.

Conclusion:

Different bacteria are the main causative agents of conjunctivitis in neonates and children under five years of age. Among the gram positive cocci, *Staphylococcus aureus* is the most common cause. *Pseudomonas aeruginosa* is the most common gram negative bacilli. chloramphenicol is the most effective antibiotics against both gram positive and gram negative bacteria. vancomycin is most effective against gram positive cocci and amikacin is most effective against gram negative bacilli. Therefore, determining the susceptibility pattern of these pathogens to the commonly available antibiotics is of utmost importance in the effective management of bacterial conjunctivitis.

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