Original Research Article

NEONATAL BACTERIAL CONJUNCTIVITIS IN TERTIARY HOSPITALS IN SANA'A CITY, YEMEN

ABSTRACT

Background: Ophthalmia neonatorum (ON) is the most common eye infection occurring in the first 28 days of life. Although most of these cases are benign, some may progress to systemic complications or blindness if left untreated. Objectives: The current study was conducted with the aim of revealing the bacteriological causes of conjunctivitis in neonates and the antibiotic sensitivity pattern of these bacteria. Materials and methods: The study included all neonates at the age of 1 to 28 days presenting at the neonatal nurseries with Neonatal Intensive Care Unit (NICU) and level II care beds in three hospitals; Authority of Al-Thawra General Hospital, Al-Kuwait University Hospital and Al-Sabeen Maternity & Child Hospital in Sana'a city, Yemen. A full history was taken from each nurse and mothers of the neonates included in the study in which the findings were recorded in a predesigned questionnaire including socio demographic, maternal clinical information and therapeutic interventions. To isolate the causative agent, the conjunctival swabs were inoculated on proper media and bacteria were identified by standard microbiological methods and antibiotic resistance was done for the isolates. **Results:** 203 swabs were collected from newborns with eye discharge over a nine-month period. Positive growth rate was 51.7%, males were more affected (57.1%), 80% of affected neonates had low birth weight, 71.4% of preterm infants, and most affected infants were preterm (P < 0.01). There was a significant relationship between invasive and non-invasive mechanical ventilation with neonatal conjunctivitis (P < 0.05). Gentamicin showed good in vitro sensitivity to all bacteria isolated, Staphylococcus aureus (83%), Escherichia coli 84.6%, with P.aeruginosa it was 60%. Conclusion: The vast majority of cases of neonatal conjunctivitis were mild with a high level of occurrence, Staphylococcus aureus and Klebsiella pneumoniae were the major bacterial agents, neonatal conjunctivitis most likely to be a hospital-acquired infection. There was a significant association between phototherapy, non-invasive ventilation and incidence of neonatal conjunctivitis. Gentamicin had high activity against the bacteria isolated in this study.

Key words: antibiotic sensitivity, bacteriological causes, conjunctivitis, neonates, ophthalmia neonatorum (ON), Yemen

INTRODUCTION

Ophthalmia neonatorum (ON) also known as neonatal ophthalmia and/or neonatal conjunctivitis is the most common eye infection occurring in the first 28 days of life. Although most of these cases are benign, some may progress to systemic complications or blindness if left untreated¹. The conjunctiva of the newborn is sterile at birth but shortly becomes colonized by many microorganisms that may be either pathogenic or non-pathogenic. The conjunctiva of the newborn is susceptible to infection, not only because of low levels of antibacterial agents and proteins such as lysozyme and immunoglobulins A and G, but because the lacrimal membrane and outflow are just beginning to develop. Neonatal ophthalmia is characterized by purulent discharge of the eye and redness of the conjunctiva with or without swelling of the eyelid. However, clinical presentations of neonatal ophthalmia are not etiologically diagnostic and microbiological work with cytology, cultures and microbial sensitivities is obligatory. The choice of antimicrobial therapy is based on the findings of the laboratory ². Neonatal conjunctivitis is a global problem with incidence ranging from 0.9% to 33% in different countries. Neonatal ophthalmia is usually acquired either from the maternal reproductive system or acquired after birth from a hospital or community setting. It can be caused by chemical inflammation, bacterial infections, and viral infections, but most cases of conjunctivitis in newborns are caused by bacterial factors³.

Bacterial pathogens most frequently reported as causative pathogens are coagulase-negative *staphylococci*, *coliforms*, *pneumococci*, *Staphylococcus aureus* and *enterococci* which tend to cause mild to moderate disease ⁴. Furthermore, viral pathogens that cause mild conjunctivitis in neonates such as *rhinovirus*, *adenovirus* and *bocavirus*. In addition, *Pseudomonas aeruginosa*, *Chlamydia trachomatis*, and *Neisseria gonorrhoeae* are associated with severe conjunctival infections ⁵. Predisposing factors, which can increase the chance of a newborn developing neonatal conjunctivitis include increased shedding of these organisms into the mother's vaginal tract during the last trimester,

premature rupture of membranes, and prolonged labor. Neonatal conjunctivitis after cesarean delivery can be due to intrauterine *chlamydial* infection as a result of early rupture of membranes⁶.

Many studies have been conducted to study the health problems of infants and children in Yemen, including infectious diseases such as tetanus, protozoa, hepatitis viruses, and even eye problems such as trachoma and corneal ulcers ⁷⁻¹⁸, but there is no single study regarding Ophthalmia neonatorum prevalence, bacteriological causes, and antibiotic sensitivity pattern, in order to know the realistic recommendations for the routine prevention of the eye, which must be practiced immediately after birth, to prevent the occurrence of this dangerous infection for newborns. Therefore, the current study was conducted with the aim of revealing the bacteriological causes of conjunctivitis in neonates and the antibiotic sensitivity pattern of these bacteria.

MATERIALS AND METHODS

Study design: A prospective cross-sectional study.

Study population and study area: This study was conducted over a period of 9 months (February, 2021- October, 2021). This study included all neonates at the age of 1 to 28 days presenting at the neonatal nurseries with Neonatal Intensive Care Unit (NICU) and level II care beds in three hospitals; Authority of Al-Thawra General Hospital, Al-Kuwait University Hospital and Al-Sabeen Maternity & Child Hospital in Sana'a city, Yemen. There were no protocols for prophylaxis to neonatorum ophthalmia in the three hospitals during the period of the study.

Inclusion criteria: All babies at the age of 1 to 28 days presenting with purulent, mucoid or muco-purulent discharge either in one or both eyes.

Exclusion criteria: Hospitalized neonates under treatment.

Sample size calculation: This cross-sectional study was performed on 203 neonates (112 male and 91 female) aged 28 days. The sample size was calculated according to the following: The population of neonates attended to the tertiary hospitals in Sana'a city was 45000 per year. With expected frequency of neonatal conjunctivitis as previous report (15.8%) ¹⁹. Also, with acceptable margin of error equal to 5% at Confidence Level 95%.

Data collection: A full history was taken from <u>each</u> nurse and mothers of the neonates included in the study in which the findings were recorded <u>in a predesigned</u> questionnaire including socio demographic, maternal clinical information and therapeutic interventions.

Specimen collection: Physical examination was conducted on all study population and the severity of the conjunctivitis was detected. The specimens were taken by a trained health care personnel by using a sterile cotton swab which was moistened with sterile saline. Two conjunctival swabs were taken, one for each eye, even if the infection was only in one eye. The specimens were collected from the inferior conjunctival fornix with avoiding the eyelid border and eye lashes and were inoculated directly onto the beforehand prepared culture plates then transported in secure boxes to Al-kwait University Hospital microbiological laboratory to be processed in the same day.

Culturing of eye swabs: To isolate the causative agent, the conjunctival swabs were inoculated on proper media and bacteria were identified by standard microbiological methods ²⁰.

Antibiotic susceptibility testing: Antibiotic resistance was done using Kirby-Bauer disc diffusion methods and interpretation of antibiotic sensitivity results was done according to CLSI²¹.

RESULTS

A total of 203 swabs were collected from neonates with eye discharge over nine months. The general characteristics of all neonates in this study are shown in Table (1). Table (2) shows that the most common bacteria implicated in ophthalmia neonatorum in order of decreasing frequency were *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa*. Table (3) shows that among 105 samples (51.7%) yielded growth and no growth was detected in 98 samples (48.3%). Among this study , the affected males were 60 (57.1%) and the affected females were 45 (42.9%). The difference between the proportions of affected males and females was not statistically significant (P = 0.56). Eighty percent of affected neonates were low birth weight (The mean \pm SD weight of the neonates was 2.6 ± 0.5 kg). Out of the total number of 105 bacterial conjunctival neonates, 30 (28.6%) babies were term (gestational age greater than 37 completed weeks), while 75 (71.4%) were preterm (gestational age < 37weeks). None of the babies was post-term (gestational age greater than 42

weeks). Most of the affected neonates were premature which was statistically significant (P < 0.01). The mean age of onset was 8.8±6.7 days Most cases of neonatal conjunctivitis78 (74.3%) occurred in the first week of life while 21 (20%) presented within the second week of life .The rest of cases 5 (4.8%) and 1 (0.95)% developed in the third and the fourth week of life, respectively. Bilateral conjunctivitis was present in 117 (57.6%) of all neonates, while unilateral involvement was seen in 86 (42.4%) of the patients. Table (4) shows that among 105 neonates with conjunctivitis, 70 (66.7%) neonates were delivered vaginally and 35 (33.3%) were delivered by cesarean section. There was no relation between the delivery mode with neonatal bacterial conjunctivitis (P = 0.46). A positive history of prolonged rupture of fetal membranes (> 18 hours) was documented in 9 (8.6%) neonates with conjunctivitis, however, it was not statistically significant (p = 0.69). Moreover, no relation between the history of maternal infections of the lower genital tract with neonatal bacterial conjunctivitis (p =0.099). Table (5) showed that there was a statistically significant between invasive and non-invasive mechanical ventilation with neonatal conjunctivitis (P < 0.05). Among 105 neonatal conjunctivitis cases, 3 cases (2.9%) needed invasive mechanical ventilation and 26 cases (24.8%) needed noninvasive mechanical ventilation mainly nCPAP (nasal continuous positive airway pressure) during their stay in NICU. There was a statistically significant association between NNPT and conjunctivitis (P < 0.01). Neonates who underwent phototherapy presented an incidence rate of conjunctivitis of 59(56.2%) notably higher than among those without phototherapy criteria 46(43.8%). There was not a statistically significant association between application of Alkohl and the occurrence of conjunctivitis (P = 0.845). Among 105 neonatal conjunctivitis, 71 (67.6%) cases were applied Alkohl and 34 (32.4%) were not applied Alkohl. Table (6) shows that gentamycin showed a good sensitivity in vitro to all isolated bacteria. S.aureus was found to be sensitive to gentamycin (83%), followed by Chloramphenicol (77.9%), ofloxacin (64.4%), erythromycin (55.9%) and tetracycline (45.2%). While, Klebsiella pneumoniae was resistant to erythromycin and sensitive to gentamycin, ofloxacin, tetracycline and chloramphenicol as following (56.6%, 43.3%, 40% and 33.3%, respectively). E.coli showed sensitivity to gentamycin, ofloxacin and chloramphenicol (84.6%, 46.1%, 40% and 38.4%, respectively). Also, P.aeruginosa showed sensitivity only to gentamycin (60%) and ofloxacin (30%).

DISCUSSION

In this study, bacteria cultures were positive in 51.7% of the neonates and 48.3% of the neonates were free of demonstrable pathogenic bacteria despite the presence of conjunctivitis. Similar prevalence rates were reported from Saudi Arabia $(60\%)^{22}$ and Iraq $(69\%)^{23}$. However, higher prevalence rates $(81.5\%)^{23}$ and $(80.5\%)^{23}$, from UAE and Iran, respectively were reported $(24.25)^{24}$. This wide variation may be attributed to differences in the geographic distribution of pathogens and standards of obstetric and perinatal care. In this study, $(98)^{24}$ samples $(48.3\%)^{24}$ did not reveal any growth that might be due to other organisms that had not been researched such as Candida albicans or viruses. This negative percentage is higher than Saudi Arabia $(40\%)^{24}$ and Iraq $(31\%)^{22.23}$.

The present study shows a high percentage of *S. aureus* as the most common causative agent (56.2%) followed by *Klebsiella pneumoniae* (28.6%), *Escherichia coli* (12.4%) and less commonly by *pseudomonas auroginosa* (2.9%). Similarly, several studies reported that *Staphylococcus aureus* is the main bacterial cause of neonatal conjunctivitis from different parts of the world, including the United Arab Emirates, Iran (53.9%), Nigeria (57.1%) and Pakistan (65%)²⁴⁻²⁷. In contrast, *Enterobacter clocae* (26.32%), *Klebsiella pneumoniae* (25.36%) and *Escherichia coli* (35%) were the primary isolates from Saudi Arabia, Iraq and India, respectively ^{22,23,28}. However, the role of *Staphylococcus aureus* in neonatal conjunctivitis is controversial because it is often isolated from the eyes of asymptomatic neonates ²⁹. However, in this study only newborns with signs and symptoms of conjunctivitis were evaluated. Although prophylaxis for neonatal *Neisseria*, *gonorrhea* and *Chlamydia trachomatis*, which are usually causes of ophthalmia neonatorum, have not been diagnosed. A similar finding of zero or minimal cases of *gonococcus* and *chlamydial* conjunctivitis has been reported elsewhere^{26, 27}. This finding may indicate a rare occurrence of *gonorrhea* and *chlamydia* in the community.

In the current study, 57.1% of ON patients were males and 42.9% were females, this is similar to that reported from Iran, Nigeria and Saudi Arabia with slightly increased in male patients (54.4%)²⁵, (66.3%) ²⁶ and (51%) ²². It was also observed in this study that the majority of positive cases of bacterial cultures occurred between the lowest birth weight (80%) and premature neonates (71.4%), because these groups of neonates are more at risk as confirmed by the results of Dias *et al.*, ³⁰. This study showed a statistical significance between gestational period and the occurrence of conjunctivitis

(P < 0.01). Neonates with a positive bacterial culture were 71.4% preterm vs. 28.6% full -term. This finding is in agreement with a study by Dias et al. in Portugal ³⁰. This can be attributed to the fact that conjunctivitis may develop more frequently in premature babies where they spend a long time with their eyes closed or covered, allowing bacteria to multiply, and due to the immature lacrimal system. A functional lacrimal system produces tear components, opening and closing of the eyelids act as a pump to facilitate tear distribution across the surface of the eye and the lacrimal ducts act as a drainage system, which carries away tears, epithelial debris and bacteria³¹. It was also noted in the current study that the vast majority of 78 cases (74.3%) occurred during the end of the first week of life. This finding is in agreement with studies from Nigeria and Iran where the majority of cases occurred within the first week of life and between 1 and 12 days of age, respectively^{26,25}. The result indicates that the first week of life is the most susceptible period for conjunctivitis in infants. Also, the mean age of onset in the current study is 8.8 days indicating that ON is postnatally acquired. This study showed that there was no statistical significance between ON and the conjunctivitis wither it is unilateral or bilateral (P = 0.883). Bilateral conjunctivitis was present in 57.1% of patients, and unilateral involvement was seen in 42.9% of the patients. This result is similar to that reported by Afjeice et al., ²⁵.

In our study, many risk factors for bacterial neonatal conjunctivitis were evaluated. Some of them are maternal risk factors and the others related to the medical intervention during the stay at hospitals wither in NICU or in the nurseries. We found that there was no statistical significance between bacterial conjunctivitis and the maternal risk factors like the mode of delivery, PROM and the history of vaginal discharge (P > 0.005). These results are similar to results from India, Iran and Pakistan^{25,27,28}. This result might be due to the fact that the conjunctivitis is acquired from the hospitals as a result of contamination not from mothers.

In regarding to other risk factors which result from clinical intervention, we found that there was statistical significance in applying the invasive or noninvasive mechanical ventilation (P < 0.05). This result is similar to that reported by Borer *et al.*, ³² and Dias *et al.*, ³⁰ in which ventilation was a risk factor. The association between ON and respiratory support including nCPAP and invasive mechanical ventilation was interpreted as infants needing ventilator assistance may allow respiratory secretions to be transferred from the nasopharynx to the eyes, particularly during suctioning ³¹. Another interpretation is that the mechanical ventilation as nCPAP usually need more manipulations and operating with hands that lead contamination with skin normal or potential pathogenic bacteria ³¹.

In this present study, there was statistical significance between applying of neonatal phototherapy and the occurrence of bacterial conjunctivitis (P < 0.01). This result is similar to the results reported by Faulhaber *et al.*, [33] and Bayatmokhtari *et al.*, ³⁴. This association is attributed to the using the eye protection devices during applying of the phototherapy ³⁵. Also, the eye shield reduces the blinking which has protective effects from bacterial colonization in the conjuntival sac and lead to conjunctivitis³⁴. In our study, there was no statistical significance in application of antimony (P = 0.845). Pure kohl contains antimony sulfide and trisulfide as its main constituents. It is a traditional eyeliner and is taken from dark stone known in Arabic as "ithmed" stone³⁶. Al-kohl is used in our community to darken the eyelids and serve as cosmetics. This result comes in agreement with a study conducted in Nigeria by Isa *et al.*,³⁷.

The sensitivity patterns in this study revealed that the highest sensitivity of *S. aureus* was to gentamicin 83% which is similar to the result reported by Dias *et al.* ³⁰. In contrast, in another study in Iraq, *Staphylococcus aureus* was more sensitive to ciprofloxacin followed by chloramphenicol ²³. *Klebsiella pneumoniae* in this study had an average sensitivity of gentamicin 56.6%, however, in another study in Nigeria, *Klebsiella pneumoniae* was resistant to gentamicin but sensitive to ceftazidime ²⁶. Finally came *Escherichia coli*, which showed a high sensitivity to gentamicin (84.6%) and moderate sensitivity of *Pseudomonas aeruginosa* to gentamicin (60%).

CONCLUSION AND RECOMMENDATION

The vast majority of cases of neonatal conjunctivitis were mild with a high level of occurrence, *Staphylococcus aureus* and *Klebsiella pneumoniae* were the major bacterial agents, neonatal conjunctivitis most likely to be a hospital-acquired infection. There was no statistically significant association between maternal risk factors (prelabour rupture of membranes, presence of vaginal discharge and mode of delivery) and neonatal conjunctivitis, but there was a significant association between phototherapy, non-invasive ventilation and incidence of neonatal conjunctivitis. Gentamicin had high activity against the bacteria isolated in this study. Microbiological examinations are needed

prior to treatment for cases of NO, routine eye prophylaxis as soon as possible after delivery, regardless of whether it was delivered vaginally or by caesarean section, and health workers in neonatal units should pay attention to infection control practices in order to reduce the level of pollution.

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CONFLICT OF INTEREST

No conflict of interest associated with this work.

AUTHOR'S CONTRIBUTIONS

The first author is a master's student who did the field and lab work and wrote the thesis. All other authors participated in writing the article, reviewing the results and supervising the master's thesis, especially Prof. Ahmed Al-Joufi and Prof. Essam Al-Shamahi.

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Results

Table 1: General characteristics of the study population.

Variable	Frequency	percentage
Gender o Male o Female	112 91	55.2 % 44.8 %
Weight o Normal weight o LBW	141 62	69.5 % 30.5 %
Gestational category	88 115 0	43.4 % 56.7 % 0 %
Onset of conjunctivitis First week Second week Third week Fourth week	112 55 21 15	55.2 % 27.1 % 10.3 % 7.4 %
Mode of delivery	140 63	69 % 31 %
Mechanical Ventilation	48 37 118	23.6 % 18.2 % 58.1 %

Table 1: continued

Variable	Frequency	percentage
Neonatal phototherapy (NNPT)		
o Yes	86	42.4 %
o No	117	57.6 %
A 4' (T7 11) 1' 1		
Antimony (Kohl) applied		
o Yes	136	67 %
o No	67	33%
Eye involvement		
o Unilateral	86	42.4%
o Bilateral	117	57.6%

Table 2: The Frequency of isolated bacteria causing neonatal conjunctivitis.

The bacteria	Number of isolates	Percentage
Staphylococcus aureus	59	56.2%
Klebsiella species	30	28.6%
Escherichia coli	13	12.4%
Pseudomonas aeruginosa	3	2.9%
Total	105	100%

Table 3: Demographic data and clinical manifestations among neonates with bacterial conjunctivitis (n=105).

Variable	Bacterial conjunctival neonates=105	Non-bacterial conjunctivital neonates=98	OR	95% CI	X2	p-value
Gender o Male o Female	60 (57.1%) 45(42.9%)	52 (53.1%) 46 (46.9%)	1.2	0.7-1.2	0.34	0.55 9
Weight o LBW o Normal weight	84(80.0%) 21(20.0%)	57(58.2%) 41(41.8%)	2.9	1.5-5.4	11.3	0.00 1
Gestational period O Preterm Term	75 (71.4) 30 (28.6)	13 (13.3%) 85(86.7%)	0.1	0.03-0.13	69.8	< 0.01
Onset of conjunctivitis	78(74.3%) 21(20.0%) 5(4.8%) 1(1.0%)	34(34.7%) 34(34.7%) 16(16.3%) 14(14.3%)	2.7	1-7.4	4.1	0.04 1
Eye involvement Our Unilateral Bilateral	45(42.9%) 60(57.1%)	41(41.8%) 57(58.2%)	1.04	0.6-1.9	0.02	0.88

 $OR (Odd \ ratio) > 1 (at \ risk)$

 X^2 (Chi square) ≥ 3.7

CI: Confidence Intervals

P (Probability value) < 0.05 (significant)

Table 4: The maternal risk factors I correlation with ophthalmia neonatorum.

Parameter	Bacterial conjunctival neonates=105	Non-bacterial conjunctivital neonates=98	OR	95% CI	X2	P-value
Mode of delivery	70(66.7%) 35(33.3%)	70(71.4%) 28(28.6%)	0.8	0.44-1.4	0.54	0.46
PROM* o Present o Absent	9(8.6%) 96(91.4%)	10(10.2%) 88(89.8%)	0.82	0.32-2.1	0.16	0.69
History of maternal infections of the lower genital tract o Infected Non-infected	50(47.6%) 55(52.4%)	58(59.2%) 40(40.8%)	0.63	0.36-1.1	2.7	0.099

OR (Odd ratio) >1 (at risk)

 X^2 (Chi square) ≥ 3.7

CI: Confidence Intervals

P (Probability value) < 0.05 (significant)

Table 5: Clinical intervention and Alkohl application as risk factors among newborns with bacterial conjunctivitis (n=105).

Parameter	Bacterial conjunctival	Non-bacterial conjunctivital	OR	95% CI	X2	P-value
	neonates=105	neonates=98				
Mechanical Ventilation	3(2.9%) 26(24.8%) 76(72.3%)	45(45.9%) 11(11.2%) 42(42.9%)	0.04 2.6	0.01-0.12 1.2- 5.6	52 6.2	< 0.01 0.013
NNPT - Exposed - Non-exposed	59(56.2%) 46(43.8%)	27(27.6%) 71(72.4%)	3.4	1.9-6.1	17	< 0.01
Alkohl (antimony) - Applied - Not applied	71(67.6%) 34(32.4%)	65(66.3%) 33(33.7%)	1.0	0.59-1.9	0.038	0.845

OR (Odd ratio) >1 (at risk)

 X^2 (Chi square) ≥ 3.7

CI: Confidence Intervals

P (Probability value) < 0.05 (significant)

NNPT: Neonatal phototherapy

^{*}PROM: Prolonged rupture of membranes

Table 6: Antibiotics susceptibility to isolated bacteria.

Antibiotic	S.aureus Sensitivity (%)	K. pneumoniae Sensitivity (%)	E. coli Sensitivity (%)	P.aeruginosa Sensitivity (%)
Chloramphenicol	46 (77.9 %)	10(33.3%)	5(38.4%)	-
Erythromycin	33 (55.9%)	-	-	-
Gentamycin	49 (83%)	17(56.6%)	11(84.6%)	2(60%)
Ofloxacin	38 (64.4%)	13(43.3%)	6(46.1%)	1(30%)
Tetracycline	32 (54.2%)	12(40%)	-	-
		ilemeris	064	
		ensi, 2		
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