**Reviewer’s Comments**



**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 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,besides 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 obligatory1,2.

The choice of antimicrobial therapy is based on the findings of the laboratory2.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. Notwithstanding, 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 disease4. 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 infections5.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 ulcers7-18, 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 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.

**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 beforehandprepared culture plates then transportedin secure boxesto 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 methods20.

**Antibiotic susceptibility testing:** Antibiotic resistance was done using Kirby-Bauer disc diffusion methods and interpretation of antibiotic sensitivity results was done according to Clinical and Laboratory Standards Institute - CLSI21.**Describe the Statistical Analysis**

**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 ±SD weight of the neonates was 2.6 ±0.5 kg).Out of the total number of 105 bacterial conjunctival neonates, 30 babies - 28.6% 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). Table5 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 neonatal intensive care unit - NICU. There was a statistically significant association between Neonatal phototherapy - 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, chloramphenicol and tetracycline?? (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% and 80.5%, from United Arab Emirates - UAE and Iran, respectively were reported24, 25. 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 samples (48.3%) 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%) 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 aeruginosa* (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 cloacae* (26.32%), *Klebsiella pneumoniae* (25.36%) and *Escherichia coli* (35%) were the primary isolates from Saudi Arabia, Iraq and India, respectively22,23,28. However, the role of *Staphylococcus aureus* in neonatal conjunctivitis is controversial because it is often isolated from the eyes of asymptomatic neonates29. However, in this study only newborns with signs and symptoms of conjunctivitis were evaluated.Although prophylaxis for neonatal *Neisseria, gonorrhea* and *Chlamydia trachomatis* was not used in these three hospitals; *Neisseria gonorrhoeae* 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 elsewhere26,27. 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 agreeswith a study by Dias et al. in Portugal30. 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 agrees 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, respectively26,25. 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 byAfjeiee*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, Prolonged rupture of membranes- PROM and the history of vaginal discharge (*p*> 0.005). These dates are similar to results from India, Iran and Pakistan25,27,28. This evidence might be due to the fact that the conjunctivitis is acquired as a result during the therapy from the hospitals contamination ().

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 suctioning31. 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 bacteria31.

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 phototherapy35. 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 chloramphenicol23. *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 ceftazidime26. 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 “ON”, 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.

**ACKNOWLEDGMENTS**

The authors extend their thanks and appreciation to Al-Kiewit University Hospital in Sana'a, and the Ministry of Health and Population who provided us with space of works in the Bacteriology Department and provided us with materials.

**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.**

|  |  |  |
| --- | --- | --- |
| Variables | Frequency(%) | P~~ercentage~~(%) |
| GenderMaleFemale | 112(52.2)91 | ~~55.2 %~~44.8 % |
| WeightNormal weightLBW(?) | 14162 | 69.5 %30.5 % |
| Gestational categoryPretermTermPost-term | 881150 | ~~43.4 %~~~~56.7 %~~~~0 %~~ |
| Onset of conjunctivitisFirst weekSecond weekThird weekFourth week | 112552115 | ~~55.2 %~~~~27.1 %~~~~10.3 %~~~~7.4 %~~ |
| Mode of deliverySpontaneousVaginal deliveryCesarean section | 14063 | ~~69 %~~~~31 %~~ |
| Mechanical VentilationInvasiveNoninvasiveWithout | 4837118 | ~~23.6 %~~~~18.2 %~~~~58.1 %~~ |

**+**

**Table 1: General characteristics of the study population…continued**

|  |  |  |
| --- | --- | --- |
| Variable | Frequency(n) | Percentage(%) |
| Neonatal phototherapy (NNPT)YesNo | 86117 | ~~42.4 %~~~~57.6 %~~ |
| Antimony (Kohl) appliedYesNo | 13667 | ~~67 %~~~~33%~~ |
| Eye involvementUnilateralBilateral | 86117 | ~~42.4%~~~~57.6%~~ |

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

|  |  |  |
| --- | --- | --- |
| Bacterias | Total number of isolates tested(%) | Percentage(%) |
| *Staphylococcus aureus* | 59(56.2) | ~~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.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Variables | Bacterial conjunctival neonates(n=105) | Non-bacterial conjunctivital neonates(n=98) | OR(>1) | CI(95%) | X2(≥ 3.7) | p-value(p< 0.05) |
| GenderMaleFemale | 60 (57.1%)45(42.9%) | 52 (53.1%)46 (46.9%) | 1.2 | 0.7-1.2 | 0.34 | 0.559 |
| WeightLBWNormal weight | 84(80.0%)21(20.0%) | 57(58.2%)41(41.8%) | 2.9 | 1.5-5.4 | 11.3 | 0.001 |
| Gestational periodPretermTerm | 75 (71.4)30 (28.6) | 13 (13.3%)85(86.7%) | 0.1 | 0.03-0.13 | 69.8 | < 0.01 |
| Onset of conjunctivitisFirst weekSecond weekThird weekFourth week | 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 | 0.041 |
| Eye involvementUnilateralBilateral | 45(42.9%)60(57.1%) | 41(41.8%)57(58.2%) | 1.04 | 0.6-1.9 | 0.02 | 0.883 |

OR: Odd ratio; CI: Confidence Intervals; X2: Chi square; P: Probability value

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

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Parameters | Bacterial conjunctival neonates(n=105) | Non-bacterial conjunctivital neonate(n=98) | OR | 95% CI | X2 | P-value |
| Mode of deliveryVaginaldeliveryCesarean section | 70(66.7%)35(33.3%) | 70(71.4%)28(28.6%) | 0.8 | 0.44-1.4 | 0.54 | 0.46 |
| PROM\*PresentAbsent | 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 tractInfectedNon-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; CI: Confidence Intervals; X2: Chi square; P: Probability value; \*PROM:

**Table 5: Clinical intervention and Alkohl application as risk factors among newborns with bacterial conjunctivitis.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Parameters | Bacterial conjunctival neonates(n=105) | Non-bacterial conjunctivital neonates(n=98) | OR | CI(95%) | X2 | P-value |
| Mechanical VentilationInvasive Noninvasive None | 3(2.9%)26(24.8%)76(72.3%) | 45(45.9%)11(11.2%)42(42.9%) | 0.042.6 | 0.01-0.121.2- 5.6 | 526.2 | < 0.010.013 |
| NNPTExposedNon-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; CI: Confidence Intervals; X2: Chi square; P: Probability value; NNPT: Neonatal phototherapy

**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%) | - | - |