The newborn conjunctival flora at the post delivery 24 hours

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Abstract

Purpose: The aim of this study was to investigate the aerobic conjunctival flora of neonates and the effects of delivery type on conjunctival flora development in neonates who were born with normal spontaneous vaginal delivery (SVD) or elective caesarean section (C/S) and who were not given prophylactic antibiotic eye drops after birth.

Methods: This cross-sectional study included 95 healthy newborns. One day after the delivery, conjunctival samples were taken from newborns who were born with normal SVD or elective C/S, and not given prophylactic antibiotic eye drops after birth. Newborns with conjunctival hyperemia and discharge were excluded from study. Samples were plated in blood agar, EMB, and chocolate agar. These cultures were incubated at 37 °C for 24–48 h. Antibiotic sensitivity was evaluated using Kirby-Bauer disc diffusion method.

Results: Staphylococcus aureus (S.aureus) growth was observed in 7 (70%) and coagulase negative staphylococcus (CNS) growth in 2 (20%) out of 10 eyes with bacterial growth in 9 culture positive newborns born with C/S. Two S.aureus strains were resistant to methicillin. On the other hand, CNS growth was observed in the conjunctival cultures of 17 out of 19 eyes with bacterial growth in 16 culture positive newborns born with SVD. In 2 eyes with CNS growth, there was also S.aureus growth. The positive cultures for S.aureus were significantly higher in the conjunctival cultures of neonates born with C/S compared to neonates born with SVD, where CNS growth was significantly lower (P = 0.002). All isolates were susceptible to vancomycin, teicoplanin, and gatifloxacin. Two isolates were resistant to methicillin.

Conclusions: In deliveries with C/S, the newborn does not contact the vagina. This may result in changes of bacterial characteristic of the flora. Culture positivity for S.aureus was higher in C/S compared to SVD, which may be important in case neonatal conjunctivitis develops.

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Keywords: Newborn; Conjunctivitis; Conjunctival flora; Staphylococcus aureus

Introduction

The newborn conjunctival flora and conjunctivitis have been previously investigated.1-5 These studies have demonstrated that the microbial flora of the maternal vagina affected the flora in the early period in babies delivered via spontaneous vaginal delivery (SVD).6-7 During SVD, the newborn contacts maternal vaginal flora and secretions5; as a result of this, following the contact of the newborn and the saprophyte and/or pathogen bacteria present in the vaginal flora, newborn conjunctival flora emerges or newborn conjunctivitis develops.1,4,5,8,9 In various studies, Staphylococcus aureus (S.aureus) was detected as the most common agent isolated from bacterial conjunctivitis of newborns.1,4,8-10 Prophylactic topical eye
drops are used in many developing countries to prevent newborn conjunctivitis, whereas they are no longer used in developed countries. The conjunctival flora starts to develop after birth. The pathogenicity of these microorganisms forming the flora affects the development of conjunctivitis. Studies have shown that coagulase negative staphylococcus (CNS) is the most frequently detected microorganism in the newborn flora. Staphylococcus may also be present in the conjunctival flora as a pathogenic bacterium.

In our study, we investigated the effect of delivery types on conjunctival flora and formation of aerobic bacterial flora of newborns.

**Methods**

Ninety-five newborn babies were included in the study. Seventy-five (79%) were born with SVD, and 20 (21%) were born with caesarean section (C/S). Newborns who received prophylactic antibiotic eye drops after birth and who had discharge/hyperemia after birth were excluded from the study. The newborns who had an uneventful delivery were included in the study. The babies whose mothers had not been screened for Neisseria gonorrhoeae or Chlamydia and had not received prenatal care were excluded. If there was suspicion of maternal infection or sexual transmitted diseases (STD), babies of those mothers were also excluded. The study was approved by the Ethics Committee of Erzurum Region Training and Research Hospital according to the Declaration of Helsinki.

**Sample collection**

The conjunctival samples were collected under sterile conditions 24 h after the delivery from the lower conjunctival fornix of both eyes using sterile swab by pulling the lower eyelid. The samples were transported to the laboratory using Stuart transport growth media (Oxoid, Hampshire, UK). Each time, the conjunctival cultures were collected using the same method by the same pediatrician after an informed consent was taken from parents.

**Culturing and antibiogram**

The conjunctival samples were directly plated on blood agar, Eosin methylene blue (EMB), chocolate agar, and MacConkey agar (Oxoid, Hampshire, UK). After 24–48 h of incubation at 37 °C, the colonies formed were evaluated. Staphylococci were identified at the species level by the conventional method of Bannerman (2003) (Colony characteristics, cell morphology, and arrangement based on colony size and pigment; anaerobic and aerobic growth; the presence of clumping factor, hemolysins, oxidase, O/F test, and catalase test). Bacterial susceptibility was determined by disc diffusion in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. The following antibiotic discs used were as follows: Penicillin (10 U), vancomycin (30 µg), teicoplanin (30 µg), erythromycin (15 mcg), clindamycin (2 mcg), gentamicin (10 µg), trimethoprim-sulfamethoxazole (25 µg), tobramycin (10 mcg), gatifloxacin (5 µg), and moxifloxacin (5 µg) (Oxoid, Hampshire, UK). S.aureus ATCC 25923 and E. coli ATCC 25922 strains were used for the standard control of the growth media and the antibiotic discs.

The colonies, which were produced in MacConkey agar, were identified according to Farmer et al. The oxidase test, glucose and lactose or sucrose fermentation, gas and H2S production in triple sugar iron agar, motility and indole production in sulfide indole motility medium, citrate and malonate utilization, arginine, lysine, and ornithine decarboxylation, phenylalanine deamination, urease production, adonitol fermentation, and methyl red and Voges-Proskauer tests (Oxoid, Hampshire, UK) were done.

In order to isolate yeast and mold, Dichloran rose Bengal chloramphenicol (DRCB) agar and Sabouraud dextrose agar (Oxoid, Hampshire, UK) were used for cultivation after incubation at 30 °C for 2–7 days. The colonies formed at the end of this period were purified at the same media. Purified cultures were examined under microscope. Then the carbohydrate tests were performed. To discriminate Candida albicans isolates, Corn Meal Agar (Oxoid, Hampshire, UK) was used for cultivation and examined for chlamydospore formation.

**Statistical analysis**

Data were analyzed using SPSS (Statistical Package for Social Sciences for Windows 17.0 software). The newborn with culture positivity in at least one eye was accepted as “culture positive newborn” and statistics were performed on “culture positive newborn”. Descriptive statistical methods (mean, standard deviation, frequency) were used to evaluate the data, and Chi-square test and Fisher's exact test were used to compare the quantitative parameters. P values < 0.05 were considered statistically significant.

**Results**

Nine neonates (45%) out of 20 born with C/S were culture positive while in the SVD group, 16 neonates (21%) out of 75 babies were culture positive. In the C/S group one newborn was bilaterally culture positive, and in the SVD group, 3 newborns were bilaterally positive. The bilaterally positive newborns had similar growth in both eyes.

The growth rates of the microorganisms are given in Table 1. The newborn with at least one positive culture in one eye was accepted as culture positive newborn. Descriptive statistical methods (mean, standard deviation, frequency) were used to evaluate the data, and Chi-square test and Fisher's exact test were used to compare the quantitative parameters. P values < 0.05 were considered statistically significant.

**Table 1.** The growth ratios of microorganisms.

|           | Number of newborns/number of eyes n/N | Number of newborns with positive culture growth n (%) | Number of eyes with positive culture growth N (%) |
|-----------|--------------------------------------|-----------------------------------------------------|--------------------------------------------------|
| C/S       | 20/40                                | 9 (45.0)                                            | 10 (25.0)                                         |
| SVD       | 75/150                               | 16 (22.7)                                           | 19 (12.6)                                         |
| Total     | 95/190                               | 25 (27.3)                                           | 29 (15.2)                                         |

C/S: Caesarean section, SVD: Spontaneous vaginal delivery, n: Number of newborns, N: Number of eyes.
eye was accepted as “culture positive newborn”. There was no significant difference between the SVD and C/S deliveries with respect to positive culture numbers. The percentage of microorganisms growing in positive cultures is given in Table 2. In the C/S group, 1 Klebsiella strain was isolated in a newborn besides a S. aureus strain. Also, 2 S. aureus strains were found resistant to methicillin in the C/S group. In 2 eyes of the SVD group, S. aureus were isolated in CNS positive newborns, and in one eye, Candida albicans were also detected. The growth of S. aureus was significantly higher in the conjunctival cultures of neonates born with C/S compared to neonates born with SVD ($P = 0.002$), where CNS growth was significantly lower ($P < 0.001$) (Table 2).

Eleven out of 19 positive cultures collected from neonates in SVD group were resistant to antibiotics. Three of these cultures were resistant to a single antibiotic and 3 were resistant to 2 antibiotics, whereas 5 cultures were resistant to 3 or more antibiotics. Two S. aureus strains were resistant to 4 antibiotics. Eleven strains were resistant to erythromycin, 5 strains were resistant to trimethoprim/sulfamethoxazole (TMP-SMX) and penicillin, and 2 strains were resistant to clindamycin. One strain was resistant to gentamicin, which is commonly used as a topical eye drop. Four out of 19 samples were resistant to moxifloxacin (Table 3). Antibiotic resistance was observed in 5 isolates from newborns delivered with C/S. Two strains were resistant to erythromycin, whereas 1 strain was resistant to clindamycin and TMP-SMX. One strain was resistant to gentamicin and tobramycin. Two S. aureus strains were resistant to methicillin. No strains were resistant to moxifloxacin and gatifloxacin, which are fourth-generation quinolones (Table 3) (Fig. 1).

**Discussion**

There are various reports on the conjunctival flora of the newborn. In the early period after birth, the development of the conjunctival flora is dependent on its contamination with the maternal vaginal flora in neonates born with SVD, while it is being shaped by environmental factors in later stages in both delivery types.

CNS has been reported to be the most frequently isolated microorganism in conjunctival cultures. Accordingly, CNS growth was also observed in 18 out of 25 positive cultures (72%) in our study.

There was no significant difference between the SVD and C/S deliveries with respect to positive culture numbers. Eder et al. compared the cultures collected at the 1st h and 12th h

| Isolate number | SVD | Erythromycin | TMP/SMX | Penicillin | Clindamycin | Gentamicin | Moxifloxacin | Tobramycin | Methicillin | Vancomycin | Teicoplanin |
|---------------|-----|--------------|---------|------------|-------------|------------|--------------|------------|-------------|------------|------------|
| 1             | S.aureus | R | R | R | S | S | R | S | S | S | S | S |
| 2             | S.aureus | R | R | R | S | S | R | S | S | S | S | S |
| 3             | CNS | R | R | S | R | S | S | S | S | S | S | S |
| 4             | CNS | S | S | S | S | R | S | S | S | S | S | S |

**Table 2**
The percentage of microorganisms growing in positive cultures.

| Microorganism | C/S n (%) | SVD n (%) | $P$ value |
|---------------|-----------|-----------|-----------|
| CNS | 2 (22.2) | 16 (100) | <0.001 |
| S. aureus | 7 (77.8) | 2 (12.6) | 0.002 |
| Klebsiella | 1 (2.5) | 1 (6.25) | N/A |
| Candida | 1 (0.0) | 1 (6.25) | N/A |

**Table 3**
Antibiotic resistance of isolates in spontaneous vaginal delivery (SVD) and cesarean section (C/S) groups.

C/S: Caesarean section; SVD: Spontaneous vaginal delivery; n: Newborn.
after birth in 126 neonates born with SVD and 64 neonates born with C/S, and observed a significant increase in the number of positive cultures in neonates in the SVD group. Although the number of bacterial strains were higher in the SVD group, there was no significant difference compared to C/S group. Isenberg et al., on the other hand, obtained higher numbers of strains in cultures collected immediately after birth in neonates born with SVD compared to neonates born with C/S. In both studies, cultures were collected during the early period after the delivery. In different studies, significant differences were found between the samples collected within the 1st hour after the delivery and samples collected at 12 and 48 h after the delivery. The differences reported were attributed to the development of the flora from surroundings (mother, hospital staff, air). In our study, all samples were collected within the first hour after birth. Differences were found between samples collected before delivery. It is believed that lactobacilli were the most isolated microorganisms from the cervicovaginal samples collected before delivery. It is believed that vaginal delivery ensures the contact between the conjunctiva and lactobacilli. This, in turn, contributes to the natural development of the conjunctival flora. It has been shown that an increased number of pathogenic bacteria is observed with a reduction of lactobacilli number. Their effects against S. aureus were observed in different studies. Unfortunately, in our study, according to lack of advanced bacterial isolation techniques in our center, we could not implement a technique to show lactobacilli presence or colony formation quantity in either newborn conjunctiva or vaginal culture of the mothers. We observed a significantly high growth of S. aureus in neonates born with C/S. We thought that the underlying reason was the loss of contact with the maternal vaginal flora, and the major difference in bacterial growth between the 2 delivery types may be attributed to the effect of lactobacilli in SVD.

Prophylaxis for newborn conjunctivitis was first performed at the end of the 19th century with silver nitrate, and this prophylaxis had been used in most developed countries during 20th century. At the end of the 20th century, some of the developed countries discontinued the prophylaxis. Also, Bell et al. reported that no-prophylaxis can also be offered to parents as a choice for women receiving prenatal care and who are screened for STD during pregnancy. Recently, it was concluded that screening all pregnant women for gonorrhea and chlamydia and treatment of infected mothers are effective means of preventing ophthalmia neonatorum. A meta-analysis suggested to rethink the universal eye prophylaxis in regions with low prevalence of maternal infection. So, for different parts of world the agents used for prophylaxis and whether it is mandate and or not may vary. In this study, we presented “no prophylaxis” as a second alternative after

![Fig. 1. The distribution of resistance against different antibiotics. TMP-SMX: Trimethoprim/sulfamethoxazole; C/S: Caesarean section; NSVD: Normal spontaneous vaginal delivery.](image-url)
antibiotic prophylaxis to parents of newborns who received regular prenatal care and had been screened for STD and included those who had accepted the no prophylaxis choice.

There are limitations of this study. Firstly, the sample size was relatively small, and secondly, our inclusion criteria were strict. We excluded babies whose mothers had not been screened for N. gonorrhoeae or Chlamydia and had not received prenatal care or if there was a suspicion of maternal infection or STD. This may have caused selection bias. Also, chlamydial detection modalities were not used in conjunctival samples, which may be a drawback. Another limitation is that this study was carried out at a baby-friendly hospital in the eastern part of the country. Since the hospital mostly serves rural areas and has a low number of infected patient admission, the infection ratios and the ratio of pathogenic bacteria growing in the hospital are very low. Therefore, we believe that the methicillin-resistant Staphylococcus aureus (MRSA) isolation rate is low in samples collected from babies. In tertiary hospitals that serve a higher number of patients and that perform major surgeries of infective cases, the hospital-acquired infections and the number of pathogenic bacteria may be important in the hospital services and newborn intensive care units, which are considered the first environment of the newborn.

We believe that the conjunctivics of babies delivered with C/S are susceptible to colonization of pathogenic bacteria, and discontinuation of prophylactic antibiotics in C/S is the cause of similar S. aureus growth in the early period cultures after the delivery. Therefore, we think that this should be taken into account during the treatment of conjunctivitis in babies born with C/S.

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