Ocular flora in newborns of mothers with Prolonged Labour at the University Teaching Hospital in Lusaka Zambia.

A study carried out in part fulfilment for the degree of Master of Medicine in Ophthalmology in the University of Nairobi, Kenya.

Dr. Kasongole David

May, 2010.
DECLARATION

This dissertation is my original work, and has not been submitted for a degree in any other university.

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To my wife Iley and our little brilliant star David junior IV for bearing with me during this long absence from home.

In soul, to my late parents David senior II and Winnie-Fridah for making me who I am today.
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8. Chairperson, University of Zambia Biomedical Research Ethics committee for approving this study.

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10. Last but not least, the caring mothers that allowed me to examine their neonates.
<table>
<thead>
<tr>
<th>CONTENTS</th>
<th>PAGES</th>
</tr>
</thead>
<tbody>
<tr>
<td>DECLARATION</td>
<td>02</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>03</td>
</tr>
<tr>
<td>DEDICATION</td>
<td>04</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENT</td>
<td>05</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>06</td>
</tr>
<tr>
<td>LIST OF FIGURES/TABLES</td>
<td>07</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>08</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>09</td>
</tr>
<tr>
<td>2.0 INTRODUCTION AND LITERATURE REVIEW</td>
<td>10</td>
</tr>
<tr>
<td>3.0 RATIONALE</td>
<td>19</td>
</tr>
<tr>
<td>4.0 OBJECTIVES</td>
<td>20</td>
</tr>
<tr>
<td>5.0 METHODOLOGY</td>
<td>21</td>
</tr>
<tr>
<td>6.0 DATA MANAGEMENT AND ANALYSIS</td>
<td>27</td>
</tr>
<tr>
<td>7.0 ETHICAL CONSIDERATIONS</td>
<td>28</td>
</tr>
<tr>
<td>8.0 RESULTS</td>
<td>29</td>
</tr>
<tr>
<td>9.0 DISCUSSION</td>
<td>41</td>
</tr>
<tr>
<td>10.0 CONCLUSION</td>
<td>45</td>
</tr>
<tr>
<td>11.0 RECOMMENDATIONS</td>
<td>46</td>
</tr>
<tr>
<td>12.0 APPENDICES</td>
<td>47</td>
</tr>
<tr>
<td>13.0 APPENDIX I : MAP OF ZAMBIA: LOCATION OF LUSAKA</td>
<td>47</td>
</tr>
<tr>
<td>14.0 APPENDIX II : INFORMATION SHEET</td>
<td>48</td>
</tr>
<tr>
<td>15.0 APPENDIX III: CONSENT/AGREEMENT FORM</td>
<td>52</td>
</tr>
<tr>
<td>16.0 APPENDIX III : QUESTIONNAIRE</td>
<td>54</td>
</tr>
<tr>
<td>17.0 REFERENCES</td>
<td>57</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

Figure 1: Flow of patients .......................................................... 29
Figure 2: Maternal age distribution ............................................. 30
Figure 3: Maternal parity ............................................................ 31
Figure 4: Newborn gestational age ............................................. 34
Figure 5: Newborn sex distribution ............................................ 35
Figure 6: Culture results distribution by maternal HIV status ........ 36
Figure 7: Culture results distribution ........................................... 37
Figure 8: Association between colony counts ............................ 38
Figure 9: Spectrum of microorganism ....................................... 39

LIST OF TABLES

Table 1: Antenatal clinic profile ................................................. 32
Table 2: Newborn profile .......................................................... 33
Table 3: Sensitivity pattern of antibiotics ................................. 40
### 1.0 LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BA</td>
<td>Blood agar</td>
</tr>
<tr>
<td>BHIB</td>
<td>Brain heart infusion broth</td>
</tr>
<tr>
<td>CA</td>
<td>Chocolate agar</td>
</tr>
<tr>
<td>CNS</td>
<td>Coagulase negative staphylococcus</td>
</tr>
<tr>
<td>CS</td>
<td>Caesarian section</td>
</tr>
<tr>
<td><em>C. trachomatis</em></td>
<td><em>Chlamydia trachomatis</em></td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>MA</td>
<td>Mackonkey agar</td>
</tr>
<tr>
<td>ON</td>
<td>Ophthalmia neonatorum</td>
</tr>
<tr>
<td><em>N. gonococcus</em></td>
<td><em>Neisseria gonococcus</em></td>
</tr>
<tr>
<td>NL</td>
<td>Normal labour</td>
</tr>
<tr>
<td>OBS/GYN</td>
<td>Obstetrics and Gynaecology</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PL</td>
<td>Prolonged labour</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical package for social scientist</td>
</tr>
<tr>
<td>TRIC</td>
<td>Trachoma inclusion conjunctivitis</td>
</tr>
<tr>
<td>UNZA</td>
<td>University of Zambia</td>
</tr>
<tr>
<td>U.o.N</td>
<td>University of Nairobi</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
</tr>
<tr>
<td>UTH</td>
<td>University Teaching Hospital</td>
</tr>
<tr>
<td>VE</td>
<td>Vaginal examination</td>
</tr>
</tbody>
</table>
Title: Ocular flora in newborns of mothers with Prolonged Labour at the University Teaching Hospital in Lusaka, Zambia.

Aim: To compare the spectrum and quantity of ocular flora in neonates of mothers with prolonged labour (PL) and those of mothers with normal labour (NL) delivered at the University Teaching Hospital (UTH) in Lusaka.

Methods: This was a prospective cohort study with a sample size of 124 subjects. Newborns delivered and before discharge from UTH were enrolled in the study. We included neonates born to mothers who had PL, defined as active labour of greater than 12 hours and those of mothers who had an uneventful labour lasting less than 12 hours. Consent was obtained from the mother or guardian. The eyes of the newborns were examined. A conjunctiva smear was taken for microscopy, culture and sensitivity within 24 hours after birth.

Results: We enrolled and analysed 132 subjects in equal arms of 66 for PL and NL. Positive conjunctiva culture results were 28.8% in neonates of mothers with PL and 21.2% in those of mothers with NL (p-value 0.315). Neonates of mothers with PL were more likely to produce a positive culture result with a relative risk of 1.50 (0.68-3.33). A higher colony count was demonstrated in neonates of mothers with PL though not statistically significant (p-value 0.415). The most frequently isolated organisms in NL were Coagulase negative staphylococcus (CNS) and Staph aureus where as in PL it was E.coli and Strep viridans (co-existing) then CNS. All the organisms were sensitive to Cefotaxime and Ciprofloxacin with a high resistance shown to Chloramphenicol and Co-trimoxazole. Mothers in PL were also more likely to be on systemic antibiotics with a relative risk of 2.2 (0.6-7.9).

Conclusion: Mothers with PL increased the occurrence of ocular organisms in their newborns compared to those with NL. However, this was not statistically significant. Mothers with PL were on systemic antibiotics which may have influenced the conjunctival culture result and overall colony counts. The most effective antibiotics were Cefotaxime and Ciprofloxacin with evident resistance to Chloramphenicol and Co-trimoxazole.
2.0 INTRODUCTION AND LITERATURE REVIEW

As in other organs and systems exposed to the environment, the ocular surface is colonized by microbes which are mainly commensals. Bacteria colonizing the conjunctiva sac produce bacteriocins and inhibitory products such as lactic and acetic acid which offer the necessary competitive advantage for survival and prevent the establishment of pathogenic microorganisms. These residents induce minimal activation of inflammation and immune responses of the host. The exact microbial population of the ocular surface depends on the age of the host, the geographical location and the climate.¹

The conjunctiva sac is colonized by bacteria at birth and remains so throughout life with changes in the flora due to various factors. A very small percentage of the population has a sterile conjunctiva sac. *Staph* species and diphtheroids are the predominant organisms with anaerobic bacteria often present in 0.33%. and 3-15% of the population have a fungal flora. The microbial flora of the newborn commonly consists of *Escherichia coli, Staphylococcus epidermidis* and *Staphylococcus aureus*. Others include, *Propionibacterium acnes* and *Corynebacterium* spp. It has been noted that with advancing age, gram negative bacteria also become part of the flora. As the pattern of these organisms changes over the years, microbes known to be residents may become virulent. The risk of cross infection to other babies exists and symptoms may vary from mild to severe debilitating disease with sequelae of blindness.² ³
2.1 Neonatal Conjunctivitis (Ophthalmia Neonatorum)

Ophthalmia neonatorum (ON) is any conjunctivitis occurring within the first four weeks of life. It has a notable history and was once a major cause of blindness. The disease has been associated with a number of organisms which have varied in their relative importance over time.

The eye of an infant is more susceptible to conjunctivitis, and the disease more serious, for a number of reasons:

- The infant cornea is comparatively soft thus an attack of conjunctivitis is likely to infect and damage the cornea.
- A newborn child produces less tears and the immune responses are not developed to amicably fight infection.
- Many organisms that are pathogenic to the mothers genital tract are also pathogenic to the conjunctiva and therefore poses a great risk of conjunctivitis in the newborn.

Prior to the 1900s, the incidence of blindness as a sequelae of ophthalmia neonatorum was as high as 50%. As of today, with the introduction of antibiotic prophylaxis, the situation is fairly different. The rates have dropped to as low as 0.04/1000 live births for gonoccocal, 4/1000 live births for chlamydial ophthalmia neonatorum, in Asia and the West from as high as 10% previously with Africa being at 24%. This highlights an obvious problem on the African continent.

A study in Kenya done in 1986 showed that Neisseria gonococcus was isolated in 40% of the mothers whose neonates had ON. It also indicated that Neisseria gonococcus was found present at 9.5% and Chlamydia trachomatis at 28% in the birth canal of expectant mothers. Another study in Kenya conducted at Kenyatta National Hospital in 1984 showed figures of Neisseria gonococcus to be 5% and that of Chlamydia trachomatis being 7.5% of the sampled pregnant population.
In several regions, ophthalmia neonatorum remains to be one of the most common infections in the first month of life and can have serious systemic as well as ocular morbidity. The prevalence of this disease and its significance as a public health problem in Zambia is yet to be known.

### 2.2 Etiology

All infants are exposed to infectious agents in the birth canal whose duration of exposure is an important factor in the development of neonatal conjunctivitis.

<table>
<thead>
<tr>
<th>Causes of Ophthalmia neonatorum</th>
<th>Time of onset postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical (silver nitrate)</td>
<td>1-36 hours</td>
</tr>
<tr>
<td>Chlamydia</td>
<td>5-14 days</td>
</tr>
<tr>
<td>Neisseria gonococcus</td>
<td>24-48 hours</td>
</tr>
<tr>
<td>Bacteria (Staphylococcus, streptococcus, Haemophilus, Escherichia coli)</td>
<td>2-5 days</td>
</tr>
<tr>
<td>Virus (herpes simplex type 1 and 2)</td>
<td>3-15 days</td>
</tr>
</tbody>
</table>

The cause of the conjunctivitis is established by the clinical picture, time course and laboratory confirmation. Ophthalmia neonatorum can also be classified as either chemical or infective with reference to the origin.

The micro-organisms of major concern and therefore of public health importance include *Neisseria gonococcus* and *Chlamydia trachomatis*. This is due to the known association of these particular microbial agents with the high risk of not only predisposing to blindness but also systemic involvement that may progress to rhinitis, otitis media, pneumonitis and life threatening neonatal sepsis. Generally the risks are related to the levels of medical care at the primary level which remains a recognizable problem in the developing world.
A study conducted in Nairobi between 2001-2002 at the Kenyatta National hospital and Pumwani maternity hospitals by Trivedy et al. found *C. trachomatis* to be a common problem with isolation of the agent in 20% of the cases whereas *N. gonococcus* was isolated in 3.3%.

These findings of *C. trachomatis* were in keeping with results of a 1986 study by Klaus et al (24%) and a study by Isenberg et al in 1991-1993 (25%). This is unlike in other studies were *Neisseria gonococcus* was still a higher challenge than *C. trachomatis*.

The many other different infective micro-organisms for ophthalmia neonatorum not listed in the previous table include the following:-


*Pseudomonas species*, deserves particular mention in that infection with this organism can result in corneal ulceration and perforation.
2.3 Mode Of Transmission

Most ophthalmic infections in the neonatal period are acquired during vaginal delivery and reflect the sexually transmitted diseases prevalent in the community.\textsuperscript{15} The transmission rate of gonorrhoeae from an infected mother to her newborn is 30-50\%.\textsuperscript{16,17} Vertical transmission may play an important role in neonatal conjunctivitis as 67\% of bacteria from the infected neonates were similar to those detected in the lower genital tract and placenta of mothers, as shown in the Study conducted in Beijing by Gao.\textsuperscript{18}

In the study carried out in Kenya by Isenberg \textit{et al.}, four perinatal factors were indentified, namely: -\textsuperscript{7}

1. Maternal vaginitis
2. Presence of meconium at birth
3. Birth in a non-sterile environment and
4. Postnatal development of endometritis

Although HIV infection is usually acquired haematogenously, there have been suggestions from the literature that direct inoculation of the eye during child birth may be another portal of entry. Furthermore, infection may also be acquired postnatally from an infected parents hands, and by convention it will still be referred to as ophthalmia neonatorum for as long as infection presents within a time space of 30 days of birth.\textsuperscript{19} Besides, flies and fomites have been associated with the transmission of micro-organisms in areas with trachoma endemicy.\textsuperscript{20}

Other factors of note, such as premature rupture of membranes and prolonged labour have been implicated with regards to increasing the risk of developing ophthalmia neonatorum. This is basically based on the understanding of their close association with the increased duration of exposure of the neonates eyes to maternal vaginal flora during passage through the birth canal.\textsuperscript{21}
2.3.1 PROLONGED LABOUR

Dystocia, one of the greatest obstetrical problems, is quite common. It occurs for the most part in primiparous women, and is usually the result of inadequate uterine contraction or slow cervical dilation or both. Some conditions causing these deficiencies are cephalopelvic disproportion, multiple gestation, hydramnios, malpresentation (particularly the occiput-posterior), occluding tumors, fullness of the bladder, concealed uterine haemorrhage, delay in rupture of membranes. In addition, a nervous, worrying temperament may seriously affect the progress of labour. Of all cephalic deliveries in the United States, 8-11% are complicated by an abnormal first stage of labour. Dystocia occurs in 12% of deliveries in women without a history of prior Caesarean delivery and it may account for as many as 60% of Caesarean sections (CS). The first stage consists of a latent phase and an active phase. Diagnosis of dystocia during the former phase of labour is uncommon and likely to be an incorrect diagnosis.

The term prolonged labour is mainly applied to the prolongation of the first stage of labour beyond 12 hours. It has also been defined as labour that needs to be augmented with oxytocin due to poor progress of the first stage. The progression of labour is generally judged by two criteria:

1. The cervical dilatation
2. Descent of the presenting part

The longer it takes for labour to progress the longer the foetus is exposed to maternal vaginal flora and this too, is associated with more vaginal examinations that may contaminate the fetus with microbes or worse even bruise the facial structures hence increasing the risk of infection.

The organisms known to cause neonatal infection via the birth canal include the following:

- Group B Strep
- E.coli
- Pseudomonas aeruginosa
- Neisseria gonorrhea
- Chlamydia Trachomatis
- Candida albicans
- Mycoplasma hominis
- Listeria monocytogenes
2.4 CLINICAL PRESENTATION

This form of conjunctivitis (i.e. neonatal conjunctivitis), as noted by eyelid oedema, conjunctiva injection and frequent conjunctiva discharge, arises within 28-30 days of birth. It can be either unilateral or bilateral. Because neonates do not develop follicles, the cellular morphology of the conjunctiva reaction is not as important as in other forms of conjunctivitis.27

The clinical presentation varies with respect to the culprit pathogen in the general population, however presentation alone does not allow for the establishment of a specific diagnosis.5

Gonoccocal Ophthalmia Neonatorum

This usually occurs as early as 24-48 hours postpartum. It is associated with a purulent discharge in copious amounts, and rapid corneal involvement causing ulceration in some cases leading to corneal perforation. It is wise to consider all purulent conjunctivitis in the first few days of life as gonoccocal until proved otherwise. The rapidity with which gonococcus can penetrate the cornea has been well documented, and gonoccocal conjunctivitis presents a true ophthalmic emergency.28

Chlamydial Ophthalmia Neonatorum

It not only causes ophthalmia neonatorum but is also associated with a variety of local and systemic infections. Infection commonly presents in the first 5 to 14 days after birth with a watery discharge that later becomes purulent though less copious than in gonoccocal ophthalmia neonatorum and sometimes patients may have a pseudomembrane.10 11

Other Organisms

Several micro-organisms have been linked to the causation of ophthalmia neonatorum although the clinical picture is milder and non-specific with particular exception of conjunctivitis due to pseudomonas spp.
Chemical conjunctivitis

A conjunctiva reaction within 24hrs of birth may result from a toxic reaction to a prophylactic topical agent such as Silver nitrate drops and not be a true infection. Rarely, silver staining of the cornea has been seen.²⁷

2.5 PREVENTION

The impact of 100 years of prophylaxis in preventing and decreasing the prevalence of the blinding disease from ophthalmia neonatorum in neonates must not lead to complacency. Until predisposing conditions are eliminated, this remains a potentially significant medical challenge.²⁹

Prevention is paramount with this disease. There are two layers of prevention as depicted below:³⁰

1. Directed towards the mother

Proper prenatal care will reveal any vaginitis with resultant treatment of the mother before delivery. Studies in the United States and other regions have shown a decreased incidence of ophthalmia neonatorum in areas of widespread prenatal care. Intervention can also be instituted during delivery to mothers in high risk groups such as prolonged labour and premature rupture of membranes. The mother should also generally be educated to present for treatment if signs of any genital urinary tract appear.

2. Directed towards the child

Since the 1880s, it has been recognized that an appropriate antimicrobial eye drop applied to the eye shortly after birth will dramatically reduce the incidence of ophthalmia neonatorum. Prevention can be given to either all neonates at delivery or else to those born in non-sterile environments and also to those with meconium presence at birth.
2.6 OCULAR PROPHYLAXIS

In 1880, Crede introduced the concept of widespread prophylaxis for gonococcal ophthalmia neonatorum with 2% silver nitrate. This method is not effective against TRIC and therefore has been supplanted by agents effective against gonococcus and TRIC, such as erythromycin and tetracycline ointments.\textsuperscript{11, 32}

A study done in Kenya in 1995 showed that 2.5% Povidone-iodine ophthalmic solution is more effective than Silver nitrate or Erythromycin eye ointment and it is less toxic and far less expensive.\textsuperscript{33}

In a more recent study in Kenya conducted at Kikuyu Eye Clinic, Isenberg \textit{et al} tried to compare the double application of 2.5% Povidone-iodine within the first postnatal day with a single drop applied at birth. However the results revealed no difference.\textsuperscript{4}
3.0 RATIONALE

Regardless of our knowledge that ophthalmia neonatorum is one of the most common infections of the newborn, many questions still remain as to when, whom and how to administer ocular prophylaxis.

It has been established that ocular floras of the neonate varies from one geographical region to the next and from time to time too, as do the micro-organisms implicated in ON. This leads to the need for an understanding of ocular surface floras in realising whether to institute ocular prophylaxis or not, based on the pathogenicity of the micro-organisms.

There are no studies conducted in Zambia to determine the nature of this flora and the associated perinatal risks factors such as prolonged labour. In addition, ocular prophylaxis of any form is not routinely performed in hospitals in Zambia. It is with this in mind that this study has been designed in an attempt to determine whether the ocular floras of neonates born to mothers with prolonged labour differs and to what degree from that of mothers with uneventful deliveries.

This data will be used for advocacy and sensitization of policy makers, health personnel and the public on the need for selective or where possible compulsory ocular prophylaxis. The results will also provide a baseline for future research works.
4.0 OBJECTIVES

4.1 Main

To compare the spectrum and quantity of ocular floras in neonates born from mothers with prolonged labour and those from mothers with uneventful delivery.

4.2 Specific

1. To compare the number of colonies of ocular floras in neonates of mothers with prolonged labour with that of neonates of mothers with uneventful labour.

2. To determine the spectrum of organisms in neonates of mothers with prolonged labour and those of mothers with uneventful labour.

3. To determine the drug sensitivity of the commonly occurring ocular surface microorganisms in neonates of mothers with prolonged labour.
5.0 METHODOLOGY

5.1 Methods

5.1.1 Study design
Prospective Cohort case study

5.1.2 Study Population
1. Neonates of mothers who had prolonged labour
2. Neonates of mothers who had an uneventful delivery

5.1.3 Study duration
The study was carried out over a period of 4 months in the year 2010
- Data collected over three months
- Data analysis and presentation during one month

5.1.4 Study Setting
This study was done at the University Teaching Hospital (UTH) in Lusaka the capital city of Zambia whose population is approximately 1,084,703 (census 2000). It is the biggest hospital in the country located approximately 4km east of the city centre in Lusaka. The hospital is an academic medical centre/university hospital with approximately 1,846 beds and 250 baby cots. It provides a full range of primary, secondary and tertiary health and medical services on an inpatient and outpatient basis. In addition, it also serves as the country’s specialist centre receiving referrals from all over the country. The hospital supports the mission of the school of medicine in Zambia which primarily focuses on teaching and research. It also supports the
school of nursing and other teaching programs in a number of technical fields. Among the several departments that the institution houses is the department of Obstetrics and Gynaecology (OBS GYN) through which this study was conducted. The department provides services for both low cost and high cost reproductive health services. The Obstetrics wing has 6 labour wards and receives an estimate of 1800 admissions per month with 1200 deliveries.
5.1.5 Case definition

Newborns of mothers admitted to the University Teaching Hospital in Lusaka.

Prolonged labour

Neonates born to mothers having a diagnosis of prolonged labour, defined as active labour of greater than 12 hours.

Normal labour

Neonates born to mothers with a labour duration less than 12 hours and uneventful.

5.1.6 Inclusion criteria

1. Neonates of mothers diagnosed with prolonged labour.
2. Neonates born to mothers with uneventful labour.
3. Written consent from the mother or guardian.

5.1.7 Exclusion criteria

1. Very sick patients
2. Neonates not delivered at the University Teaching Hospital in Lusaka
3. Lack of consent from either mother or guardian
4. Neonates more than 24 hours old
5. Neonates that had left the hospital area of the study at any one time prior to recruitment time.
6. Neonates with manifest ocular anomalies
5.1.8 Sample size

Randomized sampling method was done. Every second expectant mother and her neonate were enrolled. We used the formula below for unmatched studies to calculate the sample.

\[ n = \frac{2PQ[z_a + z_\beta]^2}{(p_1 - p_0)^2} \] ........................... 1

then simplified to \[ n = \frac{2PQ}{(p_1 - p_0)^2} \] ........................... 2

Where:

\( n \) = the required sample size

\( p_1 = \frac{p_0 R}{1 + p_0 (R - 1)} \)

\( P = \frac{1}{2} (p_1 + p_0) \), \( Q = 1 - P \)

\( p_0 \) = Proportion exposed (57%) ¹

\( R \) denoted the Relative Risk corresponding to the smaller increase in the risk of interest. (1.5)

\( z_a \) and \( z_\beta \) are the cut off points along the X-axis of the normal probability distribution that represents probability matching the 95% confidence interval (1.96) and the statistical power of 80% (0.842).

Therefore, \( n = 123.67 \)

\( \approx 124 \) study

Therefore, a total of Arm PL = 62 and Arm NL = 62.
5.1.9 Resource personnel

The following were involved at various levels of the study:-

- Ophthalmologist
- Obstetrician
- Midwife
- Microbiologist
- Laboratory technician
- Epidemiologist/Bio-statistician

5.2 STUDY MATERIALS

- Questionnaire
- Patient information sheet and Consent forms
- Pens, pencils and erasers
- Pen torch
- Sterile disposable gloves
- Sterile disposable swab sticks
- Microbiology glass slides
- Brain heart infusion broth(BHIB)
- Petri dish agar (Chocolate, Blood, Mackonkey)
- Tetracycline eye ointment
5.3 PROCEDURE

After explaining the purpose of the study to the mother or guardian, an informed consent was obtained. Demographic data and other relevant details were obtained using a structured questionnaire (appendix III). The eyes of the newborn were examined within 24 hrs postpartum, and at the same setting a conjunctival smear was taken.

**Specimen Collection**

A sterile cotton swab stick soaked in brain heart infusion (BHI) was used to collect the sample prior to any medication being instilled if necessary. The lower eye lid was everted to expose the fornix then the swab was rolled in a medial to lateral direction. As a bed side procedure, the inoculations/smears were done by the principal investigator. Glass slides were used to make thin films for microscopy i.e. Potassium hydroxide (KOH), Gram and Giemsa stain. A different cotton swab was used for inoculation on to the prepared culture media of Chocolate agar (CA), Blood agar (BA) and Mackonkey agar (MA).

The culture media was incubated for 24 hours for enriched and differential media. If no growth was obtained at 24 hours the sample was re-incubated for up to 48 hours then otherwise discarded. A positive culture was defined as growth on any of the media that was used. The colonies were counted manually then graded as heavy (>100), moderate (50-100), light (1-50) and no growth. The commonly occurring organisms in the newborns of mothers with PL were identified and subjected to tests for drug sensitivity.
During the study, data was collected using a structured data collection tool. Data entry and analysis was done by a statistician using Microsoft Excel 2007 version and the statistical package for social scientist (SPSS) version 17 for Microsoft Windows respectively. The results are illustrated/presented in the form of flow charts, tables, histograms, bar charts, pie charts, ratios and proportions were appropriate. A p-value of less than 0.05 was considered to be statistically significant with 95 % CI.
7.0 ETHICAL CONSIDERATIONS

7.1 Ethical Approval

Prior to the commencement of this study, ethical approval was sought from the University of Zambia Biomedical Research Ethics committee. Indeed, permission was also sought from the University Teaching Hospital to access patients.

7.2 Counselling

The mother/guardian was informed that no invasive procedures were to be carried out on the neonate. Relevant information was also provided to the mother/guardian regarding the risks of neonatal ocular infections. The medications (eye drops or ointment) that were used in this study are registered in Zambia. Single use disposable swabs were utilized.

7.3 Consent

Informed consent was taken from either the mother or guardian before recruitment into the study. Consent was also sought for any relevant photo-documentation. Patients were informed that they were free to discontinue their participation in the study at any time if they so wished.

7.4 Data Confidentiality

The information collected from the patients records, including the laboratory test results was kept confidential throughout the study period.
8.0 RESULTS

Figure 1: Flow chart of participants

A total of 132 mother and baby pairs were eligible and enrolled into the two study arms as illustrated above.
The mean age was found to be 22.5 years for PL and 27.5 years for NL. This was statistically significant (p-value of < 0.001). The median age was 20.5 and 27.0 years respectively.
Among the mothers with PL 48.5 % were primiparous, whereas those from the NL group were 15.2 %. The mean parity was 1.1 and 2.2 respectively with a statistical significance of < 0.001. The median parity was found to be 1.0 and 2.0 respectively.
### Table 1: Antenatal and Labour profile (n= 132)

<table>
<thead>
<tr>
<th></th>
<th>Prolonged labour n=66</th>
<th>Normal labour n=66</th>
<th>RR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of VE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>4.2</td>
<td>2.1</td>
<td>-</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median</td>
<td>4.0</td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>0</td>
<td>53 (80.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-4</td>
<td>46 (69.7)</td>
<td>13 (19.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥5</td>
<td>20 (30.3)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Antibiotics in last two weeks</strong></td>
<td>10 (15.6)</td>
<td>5 (7.6)</td>
<td>2.2 (0.6 – 7.9)</td>
<td>0.151</td>
</tr>
<tr>
<td><strong>HIV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+Ve</td>
<td>8 (12.1)</td>
<td>27 (40.9)</td>
<td>0.2 (0.1 – 0.5)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Mothers who had prolonged labour had more vaginal examinations (VE) than those who had normal labour. They were also on systemic antibiotics although this was not statistically significant. Of the mothers with normal labour 40.9 % were found to be HIV positive as opposed to 12.1 % found in the group that had prolonged labour.
Table 2: Newborn profile (n= 132)

<table>
<thead>
<tr>
<th></th>
<th>Prolonged labour n=66</th>
<th>Normal labour n=66</th>
<th>RR 95 % Cl</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (Hrs)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>10.6</td>
<td>9.5</td>
<td>-</td>
<td>0.653</td>
</tr>
<tr>
<td>Median</td>
<td>10.0</td>
<td>9.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1</td>
<td>5 (7.6)</td>
<td>5 (7.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-9</td>
<td>25 (37.9)</td>
<td>32 (48.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-19</td>
<td>29 (43.9)</td>
<td>23 (34.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 20</td>
<td>7 (10.6)</td>
<td>6 (9.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mode of Delivery</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SVD</td>
<td>64 (97.0)</td>
<td>66 (100.0)</td>
<td>-</td>
<td>0.154</td>
</tr>
<tr>
<td>C/S</td>
<td>2 (3.0)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Meconium</strong></td>
<td>2</td>
<td>4</td>
<td>0.48 (0.09-2.74)</td>
<td>0.340</td>
</tr>
</tbody>
</table>

The mean age at the time of conjunctival smear were 10.6 hours for PL and 9.5 hours for NL but was not statistically significant (p-value 0.653). In the PL group, all but two patients delivered by spontaneous vaginal delivery. Meconium stain was observed more in newborns of mothers with NL but this was not statistically significant (p-value 0.340).
Out of the 105 term deliveries 57 were from mothers who had prolonged labour and 48 from those that had normal labour (p-value 0.052).

Of those that were pre-term, 9 were born to mothers with prolonged labour and 18 from mothers who had normal labour.
Figure 5: Sex distribution of the New Born (n= 132)

The male to female ratio was 1:1.2
Out of the 72 male neonates 27 were from mothers who had prolonged labour and 45 from those that had normal labour. The p-value was statistically significant at 0.002.
None of the neonates born to mothers who had prolonged labour with a positive HIV test had a positive conjunctiva. The p-value was 0.002
Newborns of mothers with PL had more positive culture results in comparison to those of neonates from mothers who had normal labour, although the p-value was not statistically significant.
There was no statistical significance exhibited between the newborns of the two sets of mothers (p-value 0.415).
There was no statistical significance in the spectrum of organisms between neonates of mothers who had prolonged and those who had normal labour (p-value 0.088). Virulent organisms were also isolated in conjunctiva smears of neonates of mothers who had prolonged labour.
Table 3: Sensitivity pattern of antibiotics

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Strep. Viridans (n=6)</th>
<th>Staph. Aureus (n=2)</th>
<th>Escherichia Coli (n=6)</th>
<th>CNS (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>6</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>2</td>
<td>0</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>6</td>
<td>0</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>0</td>
<td>3</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>4</td>
<td>0</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Penicillin</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>

Organisms were most sensitive to Cefotaxime and Ciprofloxacin. There was a high resistance pattern to Chloramphenicol and Co-trimoxazole.

(S) number of isolates tested for the drug
(R) number of sensitive isolates
(S) number of resistant isolates

* Antibiotic disc not used on particular organism for sensitivity test.
9.0 DISCUSSION

This study was carried out in the labour ward at the largest tertiary hospital in Zambia, the University Teaching Hospital situated in the capital city Lusaka.

In this study, we sought to compare the spectrum and quantity of ocular flora in newborns of mothers who had PL and those of mothers who had had uneventful delivery. A total of 132 subjects were enrolled who were then divided into two respective arms of 66 pairs each (mother and baby) as depicted in the flow chart (Figure 1).

The mean age of the mothers was 22.5 years for PL and 27.5 years for NL (Figure 2). The median age was 20.5 years and 27.0 years respectively. This was expected as primiparous women are younger and are more likely to have PL (p-value 0.002). Among those that were primigravida (Figure 3), 48.5 % had PL and 15.2 % of the mothers had NL. The mean parity was 1.1 and 2.2 which was statistically significant (p-value 0.001) and is comparable to other studies.¹

Mothers who had PL had more vaginal examinations (VE) intra-partum than those who had NL with respect to the duration of labour (p-value = < 0.001). Most of the mothers who had PL were on systemic antibiotics at least in the last two weeks prior to this study. Majority were being treated for urinary tract infections (UTI’s). Although this result was found not to be statistically significant it was of clinical significance.

During routine screening a high prevalence of HIV reactive mothers was observed in the NL group compared to those who had PL (40.9 % and 12.1 % respectively, p-value = <0.001).

Though this finding was statistically significant, it could have been influenced by the age at first
childbirth, and/or age at first sexual intercourse. As most of the mothers in the NL group were older this may also mean that they had a stronger history of sexual exposure than mothers in the PL group (Table 1).

In this study (Table 2), the mean age at the time of conjunctiva smear for the neonates was 10.6 hours (PL) and 9.5 hours (NL) respectively. This is unlike in most studies were the average age at time of smear was between 24 and 48 hours. All were delivered by spontaneous vaginal delivery except for two among the mothers in the PL group. Meconium stain was observed more in babies of mothers with NL but was neither statistically significant nor associated with a positive culture result.

A larger proportion of babies were born at term (79.5 %) in contrast to those born before term (20.5 %), although this was not statistically significant (Figure 4). Of those that were pre-term, 9 were born to mothers with PL and the remaining 18 to mothers with NL. The male to female ratio was found to be 1:1.2.

This study illustrated (Figure 6) more positive conjunctiva culture results in babies who were born to mothers with NL and having a positive retro-viral test during screening, in contrast to those in the PL group (p-value 0.002). This might have been a result of the higher prevalence of HIV (40.9 %) among the mothers who had NL. In a recent study by Gichuhi et al carried out in Kenya, neonatal conjunctivitis was frequently diagnosed in infants born to mothers with HIV-1 infection suggesting an increased vigilance in this group.

Babies born to mothers with PL had a higher occurrence of positive culture results in comparison to those of mothers who had NL (RR 1.50). As in other studies, this was
attributed to the long duration of exposure in the birth canal and high number of VE. The two who were born by CS had a sterile conjunctival sac. In a study by Isenberg et al., sterile conjunctival cultures were more frequent in neonates delivered by CS (66%) than in neonates delivered vaginally (20%). The number of positive culture counts were lower in this study (Figure 7) at 28.8% in PL and 21.2% in NL unlike in other studies. This may be due to the fact that age at conjunctiva smear in this study was on average less than 10 hours. Mundia et al isolated 63% of positive cultures in PL and 51% in NL, and also showed that colonization increased steadily with age. Jafferji et al, isolated 43.94% of culture positive results from the normal conjunctive flora in a Kenyan population whose median age was 46.5 years. In a more recent study by Zoga et al, a higher positive culture count of 58.7% was isolated in a sampled population whose median age was 70 years.

Not only were more organisms isolated in the conjunctiva of babies from mothers who had PL but also a higher colony count was observed in them (Figure 8). A moderate to heavy growth pattern was evident in neonates (10.5%) of mothers with PL than in those with NL (7.1%). p-value 0.415.

Despite no statistical significance (p-value 0.088) in the spectrum of organisms, virulent organisms were seen in the flora of babies of mothers with PL. Non-pathogenic organisms were mostly seen in the conjunctiva flora of infants of mothers who had NL. Coagulase negative staphylococcus (35.7%), and Staphylococcus aureus (28.5%) were frequently isolated in babies from the NL group. Strep viridans and E.coli (in co-existence, 21.1%), and CNS (26.3%) were the most isolated organisms in the flora of babies in the PL group. Among the invasive organisms, Candida was found in 10% of the neonates of mothers who had PL. In the study by Mundia et al. Staph aureus, Staph epidermidis and E.coli were frequently isolated in the
conjunctiva of newborns of mothers with either NL or PL. Prentice et al had similar findings with the most isolated organisms being Staph aureus (8 %), Strep viridans (16%) and E.coli (5.4%). This was in a study in which the incidence of ON was found to be 8.2 %. In addition, Isenberg et al also illustrated the conjunctival flora of babies born by SVD to be mainly CNS (20%) followed by E.coli and Staph aureus (2 % each).

Microorganisms isolated showed resistance to Chloramphenicol and Co-trimoxazole but were more sensitive to Cefotaxime and Ciprofloxacin (Table 3). The commonly used topical drugs by general practitioners at UTH as first line include Gentamycin and Penicillin (fortified). These were not widely used for routine sensitivity tests in this study with regard to their resistance. Jafferji et al. demonstrated that Cephalosporin’s had good sensitivity whereas Gentamycin and Ampicillin had a high resistance. Mundia et al also demonstrated a similar resistance pattern.

This study had several strengths and limitations. The strengths included the prospective method of data collection, ability to perform microbiological studies on the samples in time so well as to determine the sensitivity pattern. Unfortunately, we were unable to carry out PCR for accurate isolation of organisms such as Chlamydia or Gonococcus. Colonies would have also been preferably counted using an automated machine rather than the manual method that was adopted. We were not able to collect information on the time of rupture of membranes in mothers with either PL or NL.
10. CONCLUSION

1. Prolonged labour did not result in high colony counts.

2. Mothers with PL were on systemic antibiotics which may have influenced the overall colony count.

3. A higher spectrum of organisms was isolated in babies of mothers with PL in contrast to neonates of mothers with NL. PL also increased the occurrence of pathogenic organisms in the flora of the babies.

4. Cefotaxime & Ciprofloxacin were most sensitive to the organisms isolated with Chloramphenicol and Co-trimoxazole being most resistant.
11. RECOMMENDATIONS

1. Follow up study of neonates born to mothers with PL in first month of life is needed. This is to ascertain the occurrence of ON and the causative microbial spectrum, and to see if the pattern is similar or not to that isolated within 24 hours postpartum.

2. Use of Ciprofloxacin or Cefotaxime in ON as sensitivity results are awaited, or where microbiological facilities are not available.

3. Continued testing of antibiotics for changing sensitivity pattern in Zambia
12.1 Appendix I: Map of Zambia; Location of Lusaka
Title of study: Ocular flora in newborns of mothers with prolonged labour at the University Teaching Hospital in Lusaka, Zambia.

Principal Investigator: Dr Kasongole David, MB.ChB (N.Novgorod). M.Med (Ophth) resident University of Nairobi. Kenya.

Introduction
You are being invited to participate in this aforementioned study. The purpose of our study is to determine the spectrum and quantity of ocular flora isolated from neonates of mothers who had prolonged labour and compare with that of neonates of mothers with normal labour.

Explanation of procedure
Once you accept to have your baby take part in this study you (mother or guardian) will be interviewed using a laid out set of questions. The baby's eyes and ocular adnexa will then be examined after which a conjunctival smear will be taken using a standard disposable swab stick. The specimens obtained will immediately be sent to the microbiology laboratory for specific examination.

Risks and discomforts
The procedures to be encountered are non-invasive, and therefore minimal side effects are expected if any at all. The baby is not expected to experience pain as the smear is taken.
Benefits

Any ocular infections or abnormalities present shall be addressed or otherwise relevant referral for specialist care will be made. You will also be making a major contribution to the information on ocular flora and in particular prolonged labour as a risk factor of ophthalmia neonatorum.

Confidentiality

All information gathered from the study and your identity will remain confidential throughout the process. The results may be published for planning and awareness to others.

Withdrawal without Prejudice

Enrollment into this study is voluntary and therefore refusal to participate will attract no penalty. You are free to withdraw consent and discontinue participation in the project at any time without prejudice from this institution.

Costs and/or Payments to Subject for Participation in Research

Participants will not be paid to participate in this research project.

Questions

If case of any problem or questions concerning this study, your rights as a participant, or about any research related injury, contact the principal investigator of this study: Dr Kasongole David

Telephone number: +260-979-156854 or via E-mail: kaydee133@yahoo.co.uk

or

The Chairman of the Research and Ethics Committee:

Telephone number: +260-1-256067 , E-mail: unzarec@unza.zm

Postal address: Ridgeway Campus, P.O. Box 50110, Lusaka, Zambia
12.2.1 Appendix II (Nyanja):

kudwala maso kwa ana achichepele chifukwa cha kuchedwa kubeleka kwa azimai awo pa hipatala cha University teaching Hospital Lusaka Zambia.


Mauounikila zamkati
Mwaitanidwa kutengako mbali ku mapunziro amene a chulindwa pamwamba. Cholina chamapunziro awa ndi ku peza ngira ndi muyeso wa kudwala maso kusiyana ndi azimai uchedwa kubala ndi azimai obala pa ntawi yake.

Kumasulira kwa mundondomeko
mukavomereza kuti mwana wanu atengeko mbali ku mapunziro atu (amai osunga mwana) muzafunsindwa mafunso kulingana ndi mundondomeko wa mafunso manso amwana ndi kutengamo mantogo yamene tiza peleka ku makina yopimira ndi kuyapima mantogo.

Chiyopwezo ndi kusowa mutedere
Mundondomeko wa kupunzira kwatu kulibe choipa chilichonse chingachitike ndiponso mwana wanu sazavera zowawa zilizonse potanga mantongo.

Pindu
Ngati maso ya mwana yali namatenda yamaso olo mulikalikonse kamene sikafunikika kukalamo. mwana azaonedwa ndi adotolo a maso. Muzatandizira pa kupeleka utega wa kudwala maso kwa ana chifukwa cha kuchedwa kwa kubeleka kwa amai awo.

Kusunga chisinsi
Zamene zizakambidwe ndi zi zindikilo zanu zizankala chisinsi kuyamba nikusiliza kwa mapunziro yathu. Zotuluka zizaulusidwe ndi kukonzedwa kupunzisilamo azmai ena.
Utengako mbali sikwachikakamizo ndipo ngati si mufuna ku tengakombali sitizaku leseni lugaleke pomwe mwafunira.

* kutengo/ Malipilo po tengako mbali mu kufufuzu kwatu

Tengako mbali sazalipila ndalama pa chifufu zo chatu.

* lafunso

Ngati pali zovuta ndi mafunso yokuza mapunziro yatu. Ufulu wanu otengako mbali, olo ngati wa peseka zilonda. Zibisani ofufuza pa zamapunziro a dotolo Kasongole David

a nambala iyi: +260-0979-156854 olo pa e-mail: kaydee133@yahoo.co.uk

Olo

Akulu a zo fufuza

Pa nambala: +260-1-256067. E-mail: unzarec@unza.zm

Carera: Ridgeway Campus. P.O Box 50110

Lusaka, Zambia.
12.3 Appendix III (English): Consent/Agreement form

*Kindly print in block letters in the spaces given below.*

Your signature on this form implies that you understand the information presented, and that you accept to have your child enrolled in the study. You understand that participation is voluntary, and you may withdraw from the study at any time.

- I have read the information sheet concerning this study, (or have understood the verbal explanation).
- My questions have been answered by Dr Kasongole David
- I understand that at any time I may withdraw from this study without giving a reason and without it affecting my child's care and management.
- I agree to have my child take part in this study.

Signature of mother/guardian ............................................. Date: ....................

Or Thumb Print:

Name of Participant ............................................................

Signature of Witness ......................................................... Date: ....................

Signature of Researcher ....................................................... Date: ....................
12.3.1 Appendix III (Nyanja):

Lembani muma numbera yakulu pansi apo.

Kusaina kwanu kusunyeza kuti mwa nvesesa ndi zomwe mwauzidwa, ndi kuti mwavomereza kuti mwana wanu aonedwe maso. Mwanvesesa kuti kutengako mbalu mumapunziro nikufunakwanu ndi kuti mungaleke pa ntawi iliyonse.

- Nabelenga zones za mapunziro aya (na nvesesa mau yapakamwa).
- Mafunsoyanga ya yankidwa ndi a dotolo Kasongole David
- Nanvesesa kuti nigaleke mapunziro ntawi iliyonse. Popanda kupasa lingo ndipo sichiza sokoneza kusunga mwana wanga.
- Ndavomera kuti mwana wanga atengeko mbali mu punziro ili.

Kusainakwa mai/osunga mwana................................. siku ............... 

Olo chidindo

Dzina ya odwala.................................................................

Kusaina kwa mboni................................. siku: ............... 

Kusaina ofufuza........................................ siku: ...............
12.4 Appendix IV: Questionnaire

Ocular Flora Of Newborns From Mothers With Prolonged Labour At The University Teaching Hospital In Lusaka

8.3.1 Maternal Profile

*Tick to indicate option of choice otherwise print in block letters.*

1) Date: .........................

2) IP number: ....................... 

3) Age: .............  5) Parity.........................

6) Prolonged labour □ or Normal labour □

7) Duration of labour >12 hours . Yes □ or No □

8) Number of Vaginal exams ......................

9) Medications

   Pessary/systemic antibiotic therapy taken 2 weeks prior to delivery. Yes □ or No □

   If yes specify........................................................................................................................................

10) History of antenatal vaginal discharge . Yes □ or No □

11) Antenatal screening test results *if available indicate below*

   HIV..............................

   STI *(note type)*..............

- 54 -
12.3.2 Newborn Profile

*Tick to indicate option of choice otherwise print in block letters.*

1) Date ........................................ 2) IP number ..................

2) Age in hours......................... 3) Sex M □ or F □

4) Date of delivery ...................... 5) Time ..............................

6) Mode of delivery

   SVD □  C/S □  Assisted □

7) Meconium presence. Yes □ or No □

7) Gestational Age

   Term( >37 wk) □  Preterm ( <37 wk) □

8) Date conjunctiva swab taken ........................................

9) Ocular medications given before taking swab. Yes □ or No □

   If yes specify.................................................................

10) Ocular signs of Conunctivitis. Yes □ or No □

   *If yes tick below appropriately*

   ○ Lid oedema/swelling

   ○ Conjunctiva redness

   ○ Conjunctiva chemosis

   ○ Discharge (purulent, mucoid, watery)

   ○ Others (specify)
12.3.3 Laboratory Work Up of Newborn

Date........................................ IP number..................................

Ocular Profile

a) Microscopy findings

b) Culture (*tick in the relevant box*)  +Ve ☐ or -Ve ☐

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>24 hours</th>
<th>48 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>No colonies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organism isolated</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity pattern
13.0 REFERENCES


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