OCULAR FLORA IN NEWBORNS OF MOTHERS WITH PREMATURE RUPTURE OF MEMBRANES, AND PROLONGED LABOUR

A dissertation submitted as part fulfillment

for the degree of masters of medicine (ophthalmology),

University of Nairobi.

BY

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2001

DECLARATION

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

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To my wife Jane for her love and support, and my son Dennis who kept laughing at daddy doing his homework slow and late.

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LIST OF ABBREVIATIONS

PROM	Premature Rupture of Foetal Membranes
KNH	Kenyatta National Hospital
PMH	Pumwani Maternity Hospital
O.N	Ophthalmia Neonatorum
U.S.A	United States of America
HIV	Human Immunodeficiency Virus
CMV	Cytomegalovirus
DNA	Deoxyribonucleic Acid
PCR	Polymerase Chain Reaction
WHO	World Heath Organization
MCH	Maternal Child Health
BHI	Brain Heart Infusion
HVS	High Vaginal Swab
SVD	Spontaneous Vaginal Delivery
CS	Caesarian Section
Yr.	Year
M	Male
F	Female
M.MED	Masters of Medicine
PL	Prolonged Labour
MW	Mann Whitney-U-Test

SUMMARY

An analytical case control study was carried out at Kenyatta National Hospital and Pumwani maternity Hospital. The study aimed to determine whether premature rupture of membranes (PROM), and prolonged labour (PL) increased the rate of exposure of newborn eyes to the maternal vaginal flora and whether the exposure was related to the development of neonatal conjunctivitis.

A total of 161 patients were studied between August 2000 and March 2001, amongst whom 52 had PROM, 54 Prolonged Labour and 55 controls.

Conjunctival swabs of all the newborns were taken during the first examination, for microscopy, culture and sensitivity. Any signs of conjunctivitis were also noted. HVS was also done during the first contact with the patients. The HVS were performed on 72 mothers and subjected to microscopy, culture and sensitivity.

Conjunctival swabs, taken on average, 24 hrs after birth were positive in 63% of PL, 62% of PROM and 51% of the controls.

The rate of transmission of maternal vaginal flora to the eyes of the newborns was found to be 57% in PL, 27% in PROM and 40% in the controls. Conjunctivitis developed in 31% of newborns in PL, 27% in PROM, and 23% in the controls.

In general neonatal conjunctivitis developed more frequently in those who had positive ocular cultures and significantly so in the PROM group. Conjunctivitis also developed more frequently in PL group who also showed higher transmission rates than the controls.

Staph aureus, Staph epidermidis and *E-coli* were the most common organisms isolated in the newborn eyes in all the groups. However, whereas *Staph* species were the commonly transmitted organisms in PL and controls, *E-coli* was the most common organism in PROM.

Staph aureus and *epidermidis* were resistant to Tetracycline, Kanamycin, and Penicillins. They were sensitive to Ofloxacin, Ciprofloxacin, Gentamycin, Neomycin, Tobramycin, Augmentin, Imipenem, and Cephalosporines.

E.coli was resistant to Tetracycline, Penicillin, Erythromycin, and Clindamycin. It was sensitive to Ofloxacin, Ciprofloxacin, Aminoglycosides, Cephalosporins, Chloramphenicol, Vancomycin, and Oxacillin.

Conclusions: Neonates, even the high risk ones, did not receive ocular prophylaxis. Prolonged Labour led to higher transmission of maternal vaginal flora to the eyes of newborns. PROM and PL led to higher development of conjunctivitis. The neonates who had positive conjunctival cultures developed conjunctivitis significantly higher than those with negative cultures, in the first month of life.

INTRODUCTION AND LITREATURE REVIEW

The healthy human foetus has no resident microbal populations up to the time of its birth. It acquires on its surface or swallows or inhales an assortment of microorganisms from the mother's birth canal and these are soon reinforced by contributions from various human inanimate (and possibly also animal) sources in the newborn infants immediate environment. These organisms which find themselves in suitable environments, whether on the outer or inner body surfaces, begin to mutiply and to enter into complex competitive relationships with other potential colonizers. Within hours of birth, the infant has begun to acquire a resident microbial population. Populations are different on or in different body surfaces depending on factors that enhance the organisms to thrive.[1] These organisms, which form a normal flora in their particular environments e.g. the conjunctiva, are normally harmless, but can occasionally cause disease. The pattern of these microorganisms changes over the years, and an organism once known to be harmless becomes virulent. Spread from one baby to another is a constant risk, and an organism that may cause mild symptoms in one baby may cause a more serious infection in another. [1, 10, 20]

The microbial flora widely seen in the conjunctiva of babies born of mothers with Premature Rupture of Membranes (PROM), include *Streptococcus agalatiae*, *Streptococcus viridans*, *Staphylococcus aureus*, *Haemophilus influenza*, *Bacteroides flagilis, Bacteroides melanogenicus, Peptococcus species, Escherichia coli, Gardenella vaginalis, and Candida species.*[17]

OPHTHALMIA NEONATORUM

Ophthalmia Neonatorum (O.N) is conjunctival inflammation that occurs during the first month of life (28 days). [2, 3, 21] Before 1900, up to 50% of children had been blinded by O.N. Today with the advent of ocular prophylaxis, the situation is different. Ocular prophylaxis has brought rates to as low as 0.04 per 1000 live births for gonococcal and 4 per 1000 live births for chlamydial O.N. in USA, Asia and Europe from as high as 10% previously. Rates in Africa were shown to be 24% in the same article. [3] This is therefore still a big problem in Africa. In Kenya, a study done in 1986 indicated *Neisseria gonorrhoea* could be isolated in 40% of mothers of children with ophthalmia neonatorum. In the same study, it was shown that, *Chlamydia trachomatis* was present in 28.5% and *Neisseria gonorrhoea* in 9.5% of the pregnant mothers.[3, 8] These figures may have changed in the intervening period and it will be interesting to see how. A study in 1984 in Kenyatta National hospital (KNH) showed a little contrasting figure, of *Neisseria gonorrhoea* being 5%, and *C. trachomatis* 7.5% of the pregnant population studied.[27]
Infection rates among exposed children are high and have been shown to be between 25 and 50% in Africa.[3] In many regions, O.N. is still the most common infection in the first month of life and can have serious systemic as well as ocular morbidity.[3, 9]

AETIOLOGY

Causes of O.N can be infective or chemical in origin.[3, 10] The two microbial agents of major health importance are *Neisseria gonorrhoea* and *Chlamydial trachomatis*. The risk of blindness depends on the level of medical care available, which is still a problem in developing countries and urban slums. *Chlamydia trachomatis* was the most frequently identified cause of O.N in a prospective study done in a Nairobi maternity in contrast to a study of infants seen in the S.T.I clinic, wherein *N. gonorrhoea* was the major cause. This difference may be explained by the more severer symptoms produced by *gonoccocal* O.N which leads to early seeking of medical attention.[5, 6]

In Kenya 16% of infants with *gonoccocal* O.N. showed corneal lesions in a study done in 1986.[5] Because of the penicillinase resistance, *N.gonorrhoea* in many parts of the world may be difficult to treat, or delay of treatment may be the cause of corneal lesions or generalised disease.[3, 4] Other infective agents for O.N. include *Haemophilus species*, *Streptococcus pneumonia, Staphylococcus, Escherichia coli, Enterococcus* and *Herpes simplex.* [2, 3, 7, 10]

MODE OF TRANSMISSION

Most studies point out to contamination of the eyes in the birth canal during derivery as the most common mode of transmission.[2, 3] Other modes that have been implicated include, delivery in non-sterile environment, where non-sexually transmitted organisms have been seen to predominate in the neonatal units and hospital born babies.[7, 8] Flies and

formites have also been implicated in transmission of organisms to the eyes of the exposed in trachoma endemic areas.[9] A nosocomial infection, mostly with Pseudomonas has also been shown to cause neonatal conjunctivitis. [9, 11] Factors that cause or prolong exposure of the neonatal eyes to the flora of maternal birth canal, have been shown to put the newborn at higher risk of developing O.N. These factors include premature rupture of membranes and prolonged labour. [10, 12, 17]

PREMATURE RUPTURE OF MEMBRANES

Premature Rupture of Membranes (PROM) is defined as rupture of amniotic membranes causing leakage of fluid before onset of labour. PROM complicates about 250,000 live births in the U.S.A, 40,000 being preterm PROM. [17] Rupture of foetal membranes may happen at any time during labour. It becomes a problem if the foetus is premature, (PPROM), or in the case of mature foetus, if there is a prolonged period of time between rupture of the membranes and the onset of labour (prelabour rupture of membranes). Prolonged rupture of membranes is said to occur if 24 hours elapse between rupture and onset of labour. PROM occurs in approximately 10.7% of all pregnancies, 20-40% of these being PPROM.[13, 17] In 94% of cases, the foetus is mature (approx. 20% of them being prolonged PROM). 5% occur in premature deliveries, 1000-2500gm, (50% being prolonged PROM) and < 0.5% in immature foetus, <1000gm, (75% being prolonged PROM).[13] Thus, the higher the gestational age the less the likelihood of prolonged rupture of membranes. Consequently incidence of neonatal infection decreases with increasing age at which PROM occurs. Delayed induction of labour in PROM (long latency period)

is associated with increased risk of poor perinatal outcomes including sepsis.[14] Likewise, PROM that occurs before 28 wks of gestation is related to higher risk of perinatal morbidity.[16]

A study by Mikamo, Sato et al, looked at bacterial isolates from patients with preterm labour with and without PROM. They demonstrated that 72.9% of patients with PROM had bacterial vaginosis. They also showed that 41.5% of patients with bacterial vaginosis developed PROM.[15] This implicates bacterial vaginosis as a major factor in development of PROM and neonatal sepsis.[8, 15] In Kenya organisms of note in devlopment of O.N. are *Neisseria gonorrhoea* and *C. trachomatis*, which have a prevalence of 5% and 7.5% respectively in the pregnant population.[27] Rates of neonatal infections following PROM was found to be 18%. In a study done in U.S.A, microorganisms isolated from amniotic fluids of patients with PROM were similar to those isolated from vaginal cavity. Microorganisms of neonates with sepsis were also found to be similar to those in amiotic fluids.[17]

PROLONGED LABOUR

Prolonged labour is defined as labour that takes more than 12 hours. It has also been defined as labour that needs to be augmented with Oxytocin due to delay in the first stage.[18] However this may have limitations as the need of augmentation is only known after onset of labour and that not all such labours take more than 12 hours. The longer the labour takes the longer the foetus is exposed to maternal vaginal flora and, the more frequent the vaginal examinations done, that contaminate the foetal parts with microbial

agents. Frequent digital examinations may be related to increase foetal bruising of facial structures and hence increased risk of infection. In those augmented during labour, sepsis has been demonstrated to have the highest indication of neonatal morbidity.[23]

Causes of protracted active phase of labour include cephalo-pelvic disproportion, occipital posterior and transerve positions, amniotomy performed before or at onset of labour, excessive sedation or anaesthesia administered in latent phase or early in active phase, and incoordinate uterine contractions.[13]

The organisms responsible for neonatal infections via ascending the birth canal, especially after Premature Rupture of Membranes include: Group B *Streptococcus, E-coli, Pseudomonas auroginosa, Listeria monocytogenes, Mycoplasma horminis, Neisseria gonorrhea, Hepatitis B virus, Candida albicans* and *Chlamydia trachomatis*.[20] Within the first few days of life, the baby becomes colonised by bacteria which are normally harmless, but which can occasionally cause disease. The pattern of pathogenic organisms is known to change over the years. Spread from one baby to another is a constant risk, for example, mild Staphylococcal 'sticky eye' which in itself is trivial, can be a source of much more serious infections like staphylococcal pneumonia, bullous impetigo etc, in other infants soon or at a later stage.[10, 20]

CLINICAL PICTURE

Looking at large numbers of infants, the clinical picture varies according to the causative agent, this however is not necessarily true in the individual case. Therefore clinical features do not allow making specific diagnosis.[3] As a general rule, gonoccocal O.N occurs earlier after birth and produces a more purulent discharge in large quantities than chlamydial O.N. In gonoccocal O.N. corneal involvement is more frequent, ulcerations, scarring, and perforations being exceptional.[2, 3, 10, 22] The clinical diagnosis is not only made by assessment of the swelling and redness but the cornea must be inspected. Generally, gonoccocal O.N appears 3 to 7, and chlamydial O.N, 5 to 14 days after delivery, respectively, but in case of early rupture of membranes, chlamydial O.N. may be seen earlier. [2, 3, 10, 21] Studies in Kenya indicate that newborns are taken for treatment late and therefore there is no difference in age at presentation for different organisms.[3] In Chlamydial O.N, Pseudomembranes of the conjunctiva have been observed, follicles are not seen during the first weeks of life until the third month. The clinical picture of O.N. due to other microbial agents is usually milder and nonspecific. [2, 3, 10] Infants experience hyperemia, chemosis, lid oedema, and purulent or mucopurulent exudate 5 to 21 days post-partum.[10]

Chemical conjunctivitis develops during the first hours following instillation of Silver Nitrate drops. The signs are redenning and swelling of the lids, discharge and in exceptional cases, stenosis of nasolacrimal duct. Rarely, silver staining of the cornea has been observed.[3]

DIAGNOSIS.

Since the aetiology of O.N. cannot be distiguished on the basis of clinical examination alone, laboratory investigations (smears & cultures) are mandatory.[3, 10]

The gram-stain is most important for a quick differential diagnosis as, in presence of intracellular gram-negative diplococci, gonococcal O.N. can be presumed. In the absence of intracellular gram-negative diplococci, *C.trachomatis* is the most likely causative agent. Additionally, cultures of organism are important for isolation of the microbial agent.[3] Cultures for *gonococci* and *Chlamydia* have sensitivities of 80-95% and 70-90% respectively and specificity of 100%. Sensitivity of gram stain is 90-95% in symptomatic gonococcal infections and specificity of 97-99%. Other tests available particularly in experimental set-ups are monoclonal antibody tests, DNA probes, enzyme immunoassays and polymerase chain reaction (PCR), for *N.gonorrhoea* and *C.trachomatis*.[22]

MANAGEMENT

Gonococcal ophthalmia neonatorum

Because of the seriousness of the disease, and the possible complications, and because a gram-stain of a conjuctival smear is highly predictive, cheap and easily feasible to make a presumptive diagnosis of gonococcal O.N. it can be used to direct the choice of treatment immediately the child presents.[3] However it is mandatory that a microbiological culture should be done to make a definitive diagnosis of the microbial agent and its antimicrobial

sensitivities.[3, 24,25] The recommended treatment required for *gonococcal* O.N. include multiple doses of intravenous Penicillin with or without topical antimicrobial therapy, and hospitalization.[2,3,10] Patients are hospitalised for observation of systemic complications.[10] These regimens, are however, only valid in areas where *gonococci* are not resistant to Penicillin. Upto 50% of *gonococcal* strains isolated from infants with O.N. have been reported to be penicillinase producing.[3, 26] In addition intrinsic resistance to Penicillin not mediated by penicillinase is also increasing. A single dose treatment given to the infant, as an outpatient, would be better adapted to the third world countries. These have been shown to be highly effective in areas with a high prevalence of Penicillin resistant *gonococci*.[3] For areas with penicillinase producing strains of more than 1%, W.H.O recommends O.N. should be treated with Cefotaxime 100mg/kg.[2, 3, 10] or Kanamycin 25mg/kg as a single dose intramuscularly, with 1% Tetracycline or 0.5% Erythromycin ointment for 10 days. A single dose of Kanamycin with topical Gentamicin ointment for 7 days also has been shown to be efficient.[3] Ceftriaxone has been successfully used on gonococcal O.N. without combination with topical antibiotics. [3, 10]

Chlamydial ophthalmic neonatorum

All the above regimes do not adequately treat concomitant chlamydial infections. Therefore a mixed infection treated with these regimes can result in post-gonococcal conjunctivitis due to *C. trachomatis*. Infants with O.N. with negative gonococcal laboratory tests with presumed or definitive chlamydial O.N can be treated with Erythromycin estolate 50mg/kg/day for a minimum of 14 days or another derivative of

Erythromycin with adapted dosage. Systemic treatment is unnecessary. Topical antibiotic therapy is not necessary and washing of eyes with saline is adequate. The mother and her partner(s) should be investigated and adequately treated. [2, 3, 10]

Other bacterial aetiologies.

Only the high level laboratories are well equipped to detect beta-lactamase producing strains of *N. gonorrhoea*, and chlamydial infections. Only in this situation a differentiation can be made between chlamydial and non-chlamydial O.N. Most non-gonococcal non-chlamydial O.N. can be treated with topical Erythromycin or Tetracycline ointment 4-6 times in a day for gram positive organisms and Gentamycin or Tobramycin 4-6 times in a day if gram negative organisms are isolated.[3, 10] Trimethoprim – polymyxin B combination, has broad spectrum activity against a range of both gram positive and gram negative organisms including *Pseudomonas*. [10]

PREVENTION

Prevention of neonatal conjunctivitis has over the years received increasing attention due to its effectiveness in reducing O.N. Contraversy may range as to who, when, how and what, to give but little doubt exists of the usefulness of prophylaxis. It is an important public health measure to prevent sexually transmitted infections and their consequences on pregnant women and the neonates. Intervention can be instituted in early or late pregnancy (especially in those complicated by PROM), during labour or after delivery

(depending largely on the high risk events that may occur during labour, like prolonged labour) in the mother or in the neonate. [3] Different preventive regimens can be applied; to all pregnant women, or all infants at the time of delivery or only high risk groups like PROM, Prolonged Labour, mothers with vaginitis, birth in non-sterile environment, presence of meconium at birth, etc. [8]

OCULAR PROPHYLAXIS IN THE NEONATE

Crede in Leipzig introduced this method in 1881. [3, 10] The original procedure had two parts, first, mechanical cleaning of the newborn eyelids as soon as the eyes opened, and secondly instillation of 2% aqueous silver nitrate. This was later reduced to 1% due to its irritating effect. [10]

This preventive measure was very effective and within a few years the incidence of gonococcal O.N (and blindness by it) fell from 10% to 0.3% of births.[3, 10, 21] Later ocular prophylaxis with Silver nitrate was criticized because it caused chemical conjunctivitis and was not effective against extra-ocular infection by *gonococcus*. Eye prophylaxis was abandoned in several places and was changed to prophylaxis with 0.5% Erythromycin or 1% Tetracycline ointment. [3, 8, 10, 28] Recently it has been shown that 1% Tetracycline ointment and 1% Silver Nitrate drops were equally effective prophylactic agents against gonococcal O.N. caused by multiresistant *gonococci* including penicillinase producing *N. gonococci*. However both drugs are not effective against chlamydial conjunctivitis. [3, 10] Recent studies on Povidone iodine and Povidone iodine liposomes indicate their superior effect on chlamydial infections as well as in other microbial agents. [8, 22, 29, 30] However the

need for prevention of chlamydial O.N is less clear-cut. Arguments are that chlamydial pneumonitis develops in 20% of infected infants and may be a cause of long term pulmonary problems, that untreated, *C. trachomatis* O.N may progress to scarring, corneal vascularisation and panus formation, and that the diagnosis is difficult and treatment long and costly. To prevent O.N by both organisms, 1%Tetracycline ointment or Povidone iodine could be used as first choice in countries like Kenya where prevalence of maternal *gonococci* and chlamydial infection is high, and medical facilities underdeveloped. [3, 8, 30]

RATIONALE

Although infective conjunctivitis is the commonest infection in the newborn, controversy still reigns on whom, when and how to institute ocular prophylaxis. Ocular Florae in the conjunctiva of the newborn differ from region to region and from time to time, as are the pathogens that cause neonatal conjunctivitis. Knowledge of this flora is therefore prudent in determining whether to give ocular prophylaxis or not, depending on the pathogenicity of the organisms isolated in a particular set up or region.

Some perinatal risk factors amongst which are PROM and Prolonged Labour are known to predispose the neonate to general sepsis. Little, however, is available to show whether these factors predispose the neonate to conjunctivitis specifically. It is therefore in the wake of these facts that this study attempted to determine whether the ocular flora of newborns of mothers with PROM and prolonged labour differed in any way from those with uneventiful deliveries and if so, their significance. This data will hopefully, serve as a basis for selective prophylaxis in situations of scarce resources.

MAJOR OBJECTIVE

To determine whether Premature Rupture of Membranes and prolonged labour increase the rate of exposure of the newborn eyes to maternal vaginal flora, and whether this exposure leads to higher risk of developing neonatal conjunctivitis.

SPECIFIC OBJECTIVES

1. To determine the rates of exposure of the newborn eyes to the maternal vaginal flora in PROM and Prolonged Labour and compare them with that of their controls.

2. To determine the rates of developing neonatal conjunctivitis in newborns of mothers with PROM and Prolonged Labour, and compare them with that of their controls.

3. To determine the spectrum of ocular flora in newborns of mothers with PROM and Prolonged Labour and compare them with that of their controls.

4. To determine the drug sensitivity patterns for the commonly occurring ocular microorganisms in newborns of mothers with PROM and Prolonged Labour.

METHODOLOGY

Target Population

Pregnant mothers who presented with PROM and Prolonged Labour and their newborn infants.

Study Population

1. Pregnant mothers diagnosed to have PROM and Prolonged Labour and their newborn infants, during the study period.
2. Pregnant mothers who had uneventful deliveries.

Study Area

Kenyatta National Hospital (KNH), and Pumwani Maternity hospital (PMH).

KNH is a busy national referral and teaching hospital based in the city. The labour ward admits about 800 to 1000 patients in a month. PMH is a busy city council- run maternity hospital located in the eastern part of the city. It is affordable and easily accessible to a great number of city and surrounding sub-urban residents. It admits over 2000 patients in a month in the labour ward.

Study Design

Analytical case control study.

Inclusion criteria

1. Newborns of mothers diagnosed with PROM.
2. Newborns of mothers with Prolonged Labour.
3. Mothers with PROM as diagnosed by Obstetrician and or midwife at the time of admission.
4. Mothers with PROM that resulted into the delivery in question
5. Mothers with Prolonged Labour as documented by obstetrician and or midwife.
6. Consent from the mother/guardian.

Exclusion criteria

1. Lack of consent form the mother/guardian.
2. Newborns that had been out of the hospital of study at any time between birth and time of recruitment.
3. Newborns born outside hospital of study.
4. Very sick patients and those that were mentally unstable.
5. Newborns with overt ocular anomalies.

Study Setting

Labour wards where a diagnosis of PROM was made at admission and postnatal wards where the mothers diagnosed to have PROM and Prolonged Labour were found with their newborn infants. The infants were also reviewed in the MCH clinic during the first month of life, for conjunctivitis. Mothers were, however, advised to bring the infants earlier in case they noticed signs of infection before the appointed date.

Study Period

4th Aug.2000 to 31st March 2001.

Definition of patients

Patients comprised of mothers admitted to labour wards in KNH and PMH.

Cases:

1) Antenatal mothers who presented to labour ward with preterm-PROM, defined as rupture of fetal membranes before onset of labour, before 37 weeks gestation, and their newborn infants.
2) Mothers who experienced pre-labour rupture of membranes, defined as membranes ruptured before onset of labour at term, and their infants.

3) Mothers, who experienced Prolonged Labour, defined as active labour of more than 12 hours, and their infants.

Controls:

Mothers with labour whose duration was less than 12 hours and membranes not ruptured before onset of labour, and their newborn infants. Cases were marched to controls by gestational age, mode of delivery, ward of admission, and day of delivery.

Sampling Methods

Sequential non-probability sampling method was used for the cases and controls. HVS was done on every second mother in each of the categories of PROM, Prolonged Labour, and controls. Conjunctival scrapings for PCR for *N.gonorrhoea* and *C.trachomatis* was done on every third newborn in each of the study groups.

Sample size

Formula $n = \{Z_{1-\alpha}\sqrt{[2P(1-P)]} + Z_{1-\beta}\sqrt{[P_1(1-P_1) + P_2(1-P_2)]}\}^2$

Probability of contracting disease in the cases = 50%, Controls = 20% [3]

$Z_{1-\alpha} = 0.05$, the level of significance

$Z_{1-\beta} = 0.85$, the power of the test

P = the prevalence

Sample size = **51 cases, and 51 controls**

Equipment and drugs

The following equipment was used to carry out the study; a torch, sterile disposable gloves, sterile disposable swab sticks, naked glass slides, sterile vaginal speculums, Thioglycolate broth, Brainheart infusions (BHI), chocolate agar, blood agar, MacConkay agar, Sabourauds Dextrose agar.

Erythromycin drops and Tetracycline ointment were some of the drugs included in the kit.

Resource persons

The following were the resource persons engaged to carry out the study; ophthalmologists, microbiologist, epidemiologist/Bio-statistician, obstetrician, midwives, and Laboratory Technicians

STUDY PROCEDURE

The purpose of the study and the procedure were clearly explained to the mother and informed verbal consent obtained. The eyes of the baby were then examined, and any abnormalities noted were explained to the mother.

Demographic data, relevant antenatal and perinatal history was taken and the questionnaire (appendix II) duly completed. The conjunctival and high vaginal swabs (HVS) were taken at the same sitting. The mothers were issued with a special identification card, which they presented at MCH clinics on appointed dates, approximately 10 to 14 days after delivery.

Making smears for microscopy was done as follows:

Conjunctival smears: Samples from the conjunctiva were taken by gently rolling BHI wetted swab sticks in the lower conjunctiva fornix and smearing it on naked glass slides to make thin films. The naked slides were first examined then treated with KOH, then gram and Giemsa Stained for further examination. Specimens for PCR were made with special conjunctival swabs and transported in a special container for processing in the molecular laboratory.

Vaginal Smears: Samples from vaginal fornices were also smeared on naked glass slides and stained and examined as for the conjunctival smears. To obtain the smears, the vulva was first cleaned with saline and a sterile cuscos speculum introduced into the vagina to

expose the upper vagina and the cervix. A sterile BHI wetted cotton-tipped swab stick was then introduced to obtain samples from behind the cervix.

Inoculating samples for cultures and sensitivity was done as follows:

Conjunctival Samples: A sterile BHI wetted swab stick was gently rolled in the lower conjunctiva fornix. The sample was then inoculated in the culture media in the following order: first in chocolate agar, followed by blood agar, followed by MacConkay agar, and then Sabourauds agar. The Swab sticks were then dipped in the test tube containing Thioglycolate broth. A second stick was likewise wetted in BHI and rolled in the Conjunctival fornix and the sample so obtained inoculated back to the BHI broth.

Vaginal Samples: A sterile HVS swab stick wetted in BHI was rolled in the vagina fornix. The specimen so collected was inoculated in the standard method onto the solid media and broth as for the conjunctiva specimens. Transport media were not required as inoculation of the media was done directly at bedside.

Blood and chocolate agar media were incubated in Carbon Dioxide jars at 37^0C MacConkay agar plate was incubated at 37^0 C aerobically for 24 hours. Sabourauds agar plates were incubated for 14 days at 28^0C, this being the optimal temperature for fungal growth. BHI and Thioglycolate were incubated at 37^0 C aerobically.

The types of organisms seen at microscopy were noted in the questionnaire. The culture media were read after 24 hours for the enriched and differential media and after14 days

for Sabourauds media. A positive culture was defined as growth on any of the media used. Scientific methods were used to identify species on the positive cultures.

Plates were discarded after 48 hours if no growths were obtained for all other media except Sabourauds, which was discarded after 14 days. Once the organisms from the newborn conjunctiva were identified, they were subjected to drug sensitivity. The antibiotic discs used were chosen based on the organism isolated (Kirby-Bauer method for sensitivity testing).[34]

Mothers who were found to have foul smelling per vaginal discharge or lochia received amoxicillin capsules or erythromycin tablets and flagyl while awaiting the results of culture and sensitivity. Likewise, newborns found to have signs of conjunctivitis were given chloramphenicol eye drops while awaiting the results of culture and sensitivity. Those with suspicion of gonoccocal infection received six hourly doses of systemic penicillin and topical tetracycline. Both the mother and baby were reviewed in a week to assess response to treatment. Change of antibiotic was done accordingly where no response was observed. Other ocular or systemic abnormalities seen were noted and referred appropriately.

Ethical Considerations

1. Informed consent was obtained from the mother/guardian before recruitment into the study.
2. Confidentiality of patients' records was observed.
3. Treatment and or appropriate referral were done to all deserving cases.
4. This protocol was reviewed and approved by the KNH Ethical and Research Committee.

Data analysis

Data input and analysis were carried out with SPSS for windows and Microsoft excel and standard procedures employed for data validation.

Qualitative factors between the cases and controls were analyzed using EPI- INFO, Odds ratios and Chi-square tests. Quantitave data was analyzed using the Non-parametric Mann-Whitney student U- test.

Study limitations

The study had its own limitations, which could have influenced the results in one way or another. PCR could not be done on every neonate and maternal specimens due to the high cost. The authors relied on the mothers to tell whether they or their newborns received any medications prior to examinations and taking conjunctival swabs. This was open to prejudice and heavily depended on mothers' observations and intelligence. On sensitivity

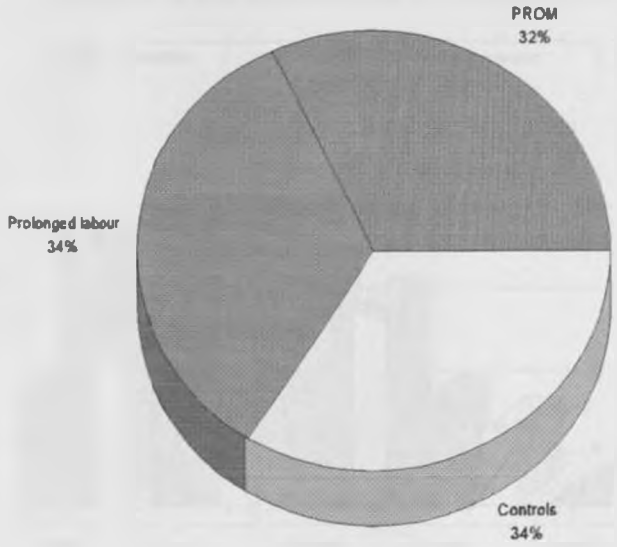
testing, not all the organisms were tested for their particular standard antibiotics, as the discs would, on occasions, be out of stock. This rendered the number of organisms tested for certain antibiotics small, and therefore precluded generalization of response for such organisms. Colony counts could not be done due to technical reasons, which made it difficult to draw conclusions on some findings.

RESULTS.

Table 1: Distribution of subjects according to study groups.

Study groups	Number of subjects	Percent (%)
PROM	52	32
Prolonged labour	54	34
Control	55	34
Total	**161**	**100**

Figure i: Distribution of subjects according to study groups

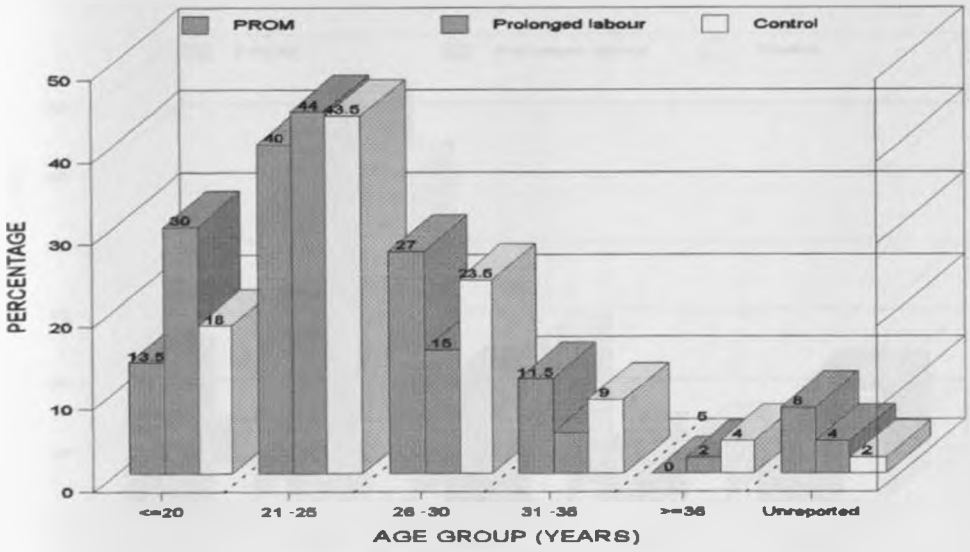


This table/figure shows distribution of subjects according to the study groups

Table 2: Maternal age distribution in the study groups.

Age (years)	PROM		Prolonged labour		Controls	
	Frequency	%	Frequency	%	Frequency	%
≤20	7	13.5	16	3.0	10	18.0
21-25	21	40.0	24	44.0	24	43.5
26-30	14	27.0	8	15.0	13	23.5
31-35	6	11.5	3	5.0	5	9.0
≥36	0	0	1	2.0	2	4.0
Unreported	4	8.0	2	4.0	1	2.0
Total	**52**	**100**	**54**	**100**	**55**	**100**

Figure ii: Maternal age distribution in the study groups.

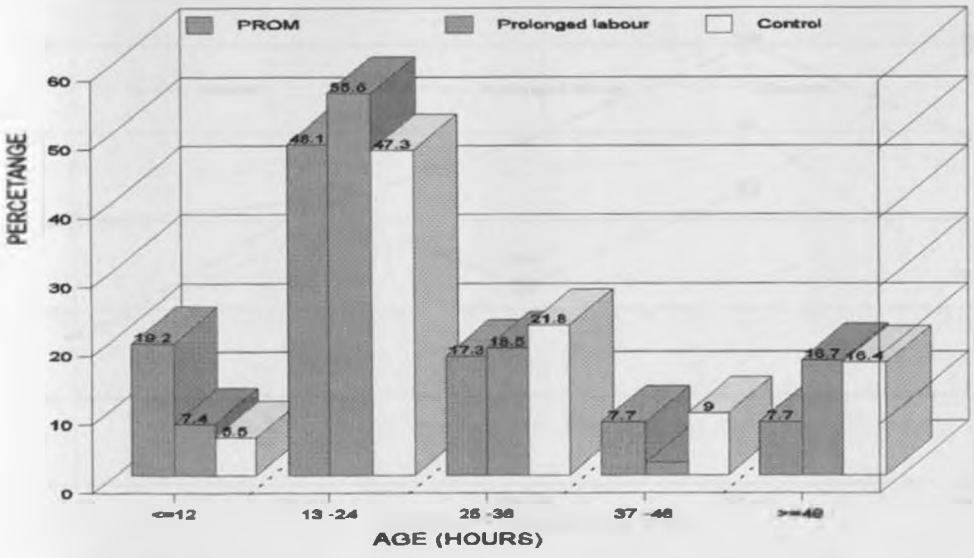


Mean age in years: PROM=25 PL=23 Controls=23
Mode in years 23 18 23
The table/figure shows how the maternal ages were distributed. They fell under normal distribution. Slightly younger patients were seen in the prolonged labour group . There was not significant difference in the mean ages (P=0.32).

Table 3: Distribution of newborns by age at which conjunctival swabs were taken.

Age (hours)	PROM (n=52)		Prolonged labour(n=54)		Controls (n=55)	
	Frequency	%	Frequency	%	Frequency	%
≤12	10	19.2	4	7.4	3	5.5
13-24	25	48.1	30	55.6	26	47.3
25-36	9	17.3	10	18.5	12	21.8
37-48	4	7.7	1	1.8	5	9
≥49	4	7.7	9	16.7	9	16.4
Total	**52**	**100**	**54**	**100**	**55**	**100**

Figure iii: Distribution of newborns by age at which conjunctival swabs were taken.



Mean: PROM=25 PL=24 Controls=25

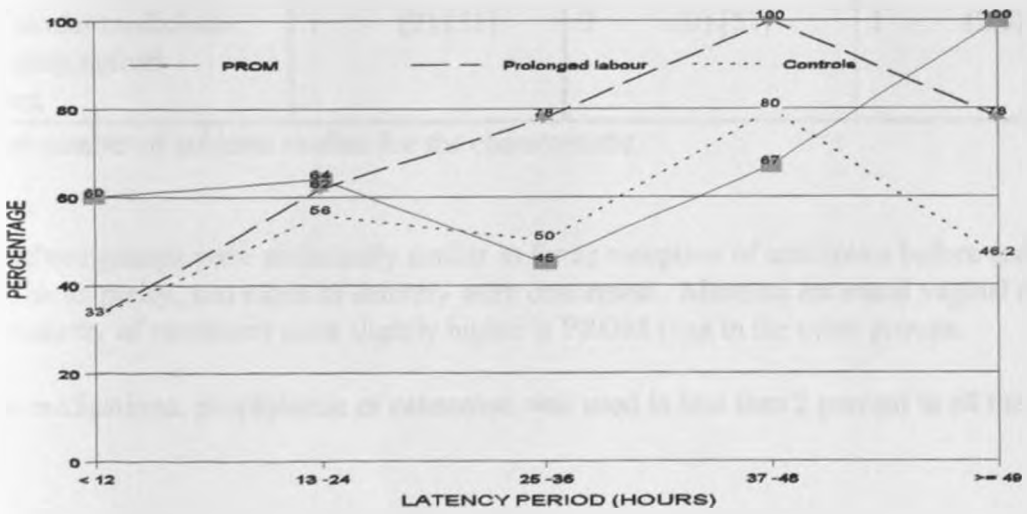
This table/figure shows the distribution of infants by their ages in hours when they were first examined and conjunctival swabs taken. On average, the infants were seen 25 hours after birth. The distribution was similar for all the groups studied.

Table 4: **Age of newborns at the time of taking conjunctival swabs versus conjunctival culture**

Age (hours)	PROM			Prolonged labour			Controls		
	No. with +ve culture		%	No. with +ve culture		%	No. with +ve culture		%
≤12	6	*[10]	60	1	[3]	33	1	[3]	33
13-24	16	[25]	64	18	[29]	62	12	[26]	46.2
25-36	4	[9]	45	7	[9]	78	6	[12]	50
37-48	2	[3]	67	1	[1]	100	4	[5]	80
≥49	4	[4]	100	7	[9]	78	5	[9]	56
Total	**32**	**51**		**34**	**51**		**28**	**55**	

*[ ] Total number of subjects studied in the age bracket.

Figure iv: Age of newborns at the time of taking conjunctival swabs versus conjunctival culture.



PROM: P value=0.443 PL:P value=0.512 Controls P value=0.666

This table/figure shows the proportions of newborns with positive ocular cultures in each of the age brackets. Higher age brackets were associated with higher proportions of positive ocular cultures. However, PROM showed high proportions even in lower age brackets.

Note that one result of culture in PROM and three in PL group were not available.

Table 5: Summary of other characteristics of patients in the study groups.

Characteristics	PROM	Prolonged labour	Controls
	Frequency (%)*[ ]	Frequency (%) *[ ]	Frequency (%) *[ ]
Mothers who received antibiotics 2 weeks before and during delivery	6 (13) [48]	7 (13) [52]	4 (7) [55]
Mothers who had antenatal vaginal discharge	7 (15) [48]	5 (10) [51]	5 (9) [54]
Mean parity of mother	2	2	2
Premature babies (< 37 weeks)	5 (10) [50]	1 (2) [53]	3 (5.7) [53]
Delivery by Caesarean section	1 (2) [51]	2 (3.7) [54]	2 3.6) [55]
Use of ocular medicines before conjunctival swabbing	1 (2) [51]	0 (0) [52]	1 (2.0) [54]

*[ ] Total number of subjects studied for the characteristic.

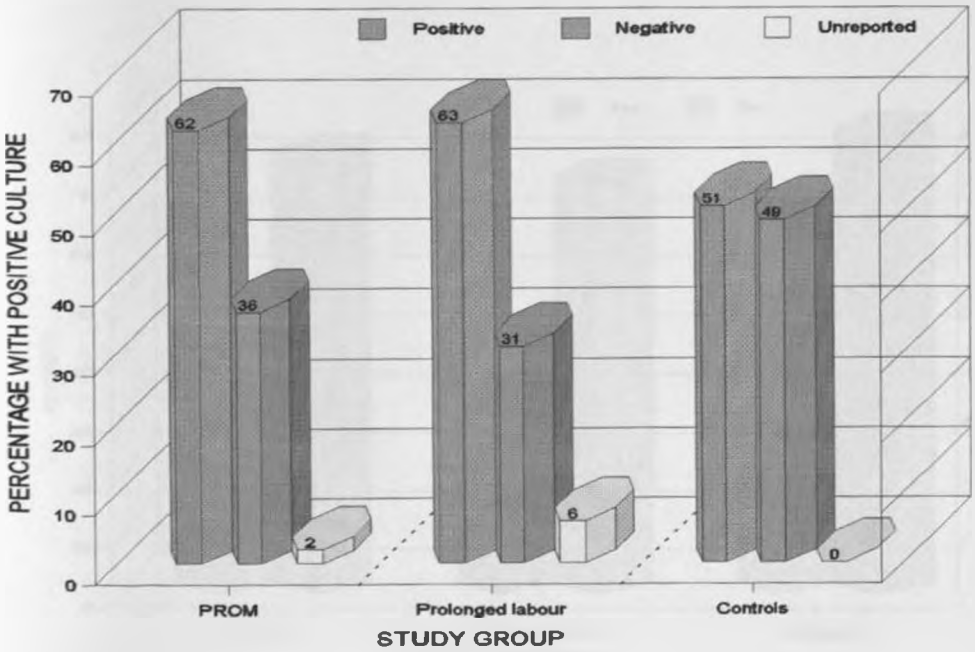
* All the three groups were statistically similar as far as reception of antibiotics before and during delivery, mean parity, and mode of delivery were concerned. Maternal antenatal vaginal discharge and prematurity of newborns were slightly higher in PROM than in the other groups.

* Ocular medications, prophylactic or otherwise, was used in less than 2 percent in all the study groups.

Table 6: Conjunctival culture distribution in study groups.

Culture	PROM		Prolonged labour		Controls	
	Frequency	%	Frequency	%	Frequency	%
Positive	32	62.0	34	63.0	28	51
Negative	19	36.0	17	31.0	27	49
Unreported	1	2.0	3	6.0	0	0
Total	**52**	**100**	**54**	**100**	**55**	**100**

Figure v: Conjunctival culture distribution in study groups.



Odds ratios: PROM=1.6 (P value=0.20) Prolonged labour=1.9 (P value=0.09)

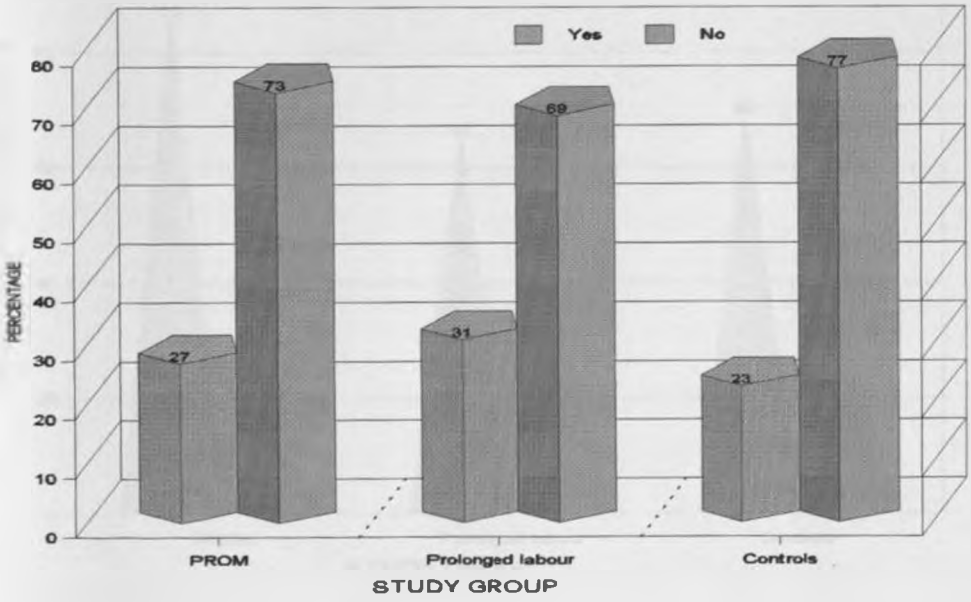
The table/figure shows how the culture results were distributed in the three study groups. There were more frequent +ve cultures than negative cultures in the PROM and PL groups, as opposed to equal frequencies in the controls.

Proportions of positive cultures in PROM=62%; Prolonged labour=63%; Controls=51%.

Table 7: Neonatal conjunctivitis distribution in the study groups.

Conjunctivitis	PROM(n=52)		Prolonged labour(n=54)		Controls(n=55)	
	Frequency	%	Frequency	%	Frequency	%
Yes	14	27	17	31	13	23
No	38	73	37	69	42	77
Total	**52**	**100**	**54**	**100**	**55**	**100**

Figure vi: Neonatal conjunctivitis distribution in the study groups.



Odds ratios: PROM=1.2 (P value=0.69) Prolonged labour=1.6 (P value=0.26)

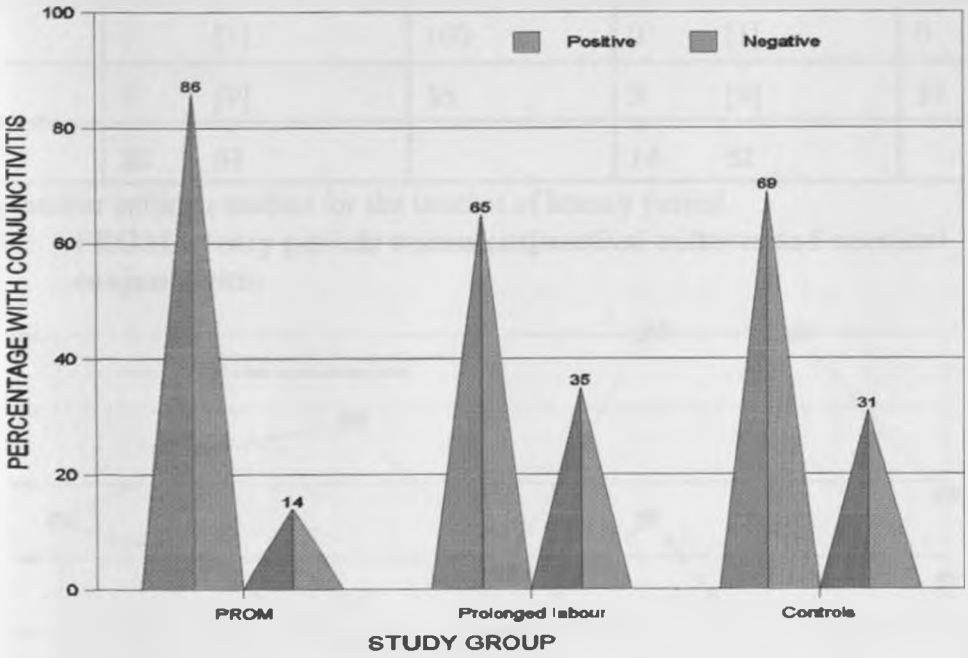
Rates of conjunctivitis in PROM=27%; Prolonged labour=31%; Controls=23%

This table/figure shows how conjunctivitis developed in the three study groups. PL and PROM had higher proportions of neonates developing conjunctivitis than the controls.

Table 8: Relationship between neonatal conjunctivitis and conjunctival culture.

Culture	PROM(n=14)		Prolonged labour(n=17)		Controls(n=13)	
	No. with conjunctivitis	%	No. with conjunctivitis	%	No. with conjunctivitis	%
Positive	12	86	11	65	9	69
Negative	2	14	6	35	4	31
Total	**14**	**100**	**17**	**100**	**13**	**100**

Figure vii: Relationship between neonatal conjunctivitis and conjunctival culture.



Odds ratios: PROM =5.00 (P value=0.037)
Prolonged labour =0.88 (P value=0.830)
Controls =2.73 (P value=0.130)

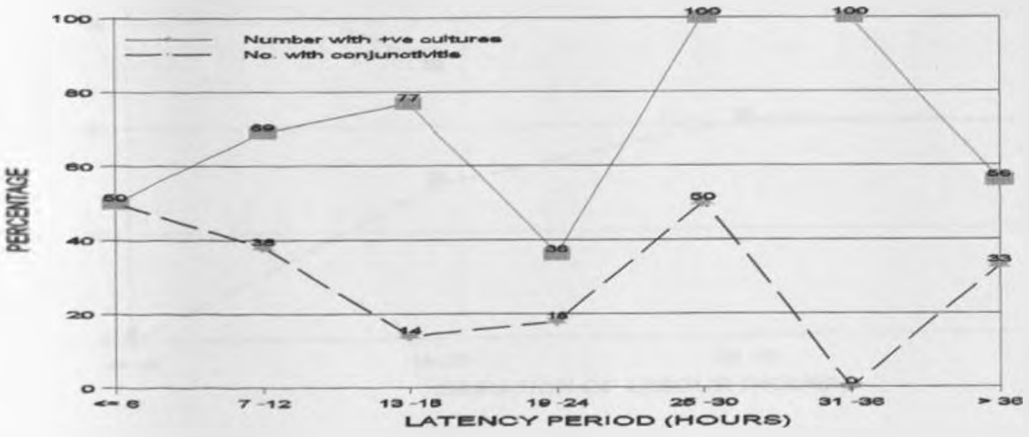
This table/figure shows the relationship between having a +ve culture and development of conjunctivitis. In all the three groups, there were higher chances of developing conjunctivitis in those with +ve culture than those with negative cultures and statistically significantly so in the PROM group.

Table 9: **PROM latency periods versus conjunctival cultures and neonatal conjunctivitis.**

Latency period (hours)	Number with positive cultures		Number with conjunctivitis	
	Frequency	%	Frequency	%
≤ 6	1 *[2]	50	1 [2]	50
7-12	9 [13]	69	5 [13]	38
13-18	10 [13]	77	2 [14]	14
19-24	4 [11]	36	2 [11]	18
25-30	2 [2]	100	1 [2]	50
31-36	1 [1]	100	0 [1]	0
> 36	5 [9]	56	3 [9]	33
Total	**32 51**		**14 52**	

*[ ] Total number subjects studied for the bracket of latency period.

Figure viii: **PROM latency periods versus conjunctival cultures and neonatal conjunctivitis.**



This table/figure shows the proportions of neonates with positive ocular cultures and conjunctivitis in each of consecutive brackets of lengths of PROM (latency periods). Longer latencies were associated with higher proportions of positive cultures.

Latency period versus +ve cultures: P=0.498 (MW test)

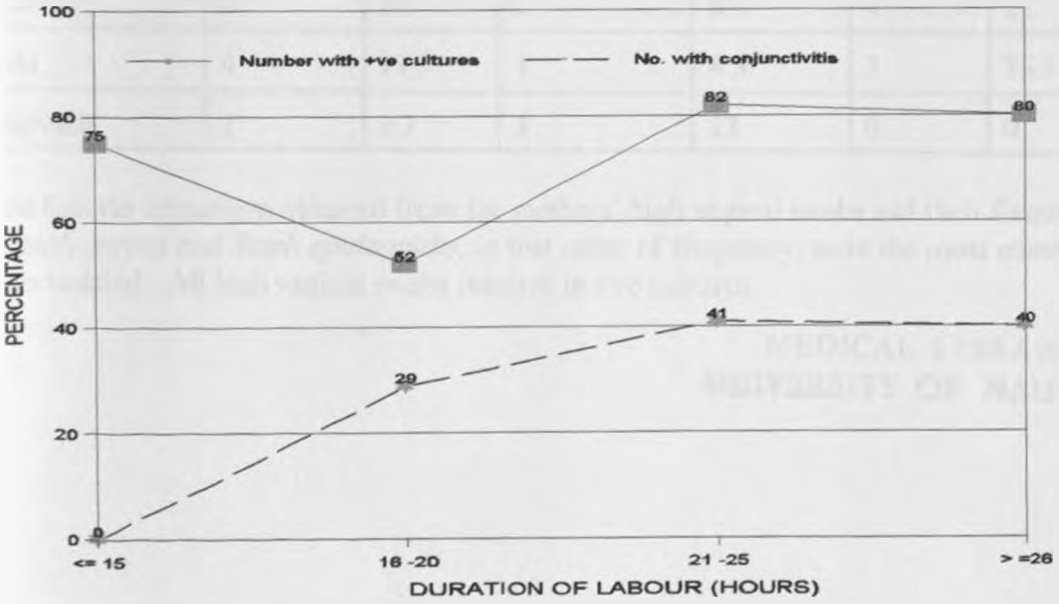
Latency period versus -ve cultures: P=0.561 (MW test).

Table 10: Duration of labour versus conjunctival cultures and neonatal conjunctivitis.

Duration of labour (hours)	Number with positive cultures		Number with conjunctivitis	
	Frequency	%	Frequency	%
≤ 15	3 *[4]	75	0 [4]	0
16-20	13 [25]	52	8 [28]	29
21-25	14 [17]	82	7 [17]	41
> 26	4 [5]	80	2 [5]	40
Total	**34 51**		**17 54**	

*[ ] Total number of subjects studied for the bracket of the duration of labour

Figure ix: Duration of labour versus conjunctival cultures and neonatal conjunctivitis.



This table/figure shows the proportion of neonates with positive ocular cultures and conjunctivitis in each of consecutive brackets of duration of labour. Long durations of labour were associated with higher proportions of positive ocular cultures and conjunctivitis.

Duration of labour versus +ve conjunctival culture : P value=0.139(MW test)

Duration of labour versus conjunctivitis: P value=0.029 (MW test).

Table 11: Profile of organisms isolated from the mothers in the study groups.

Organism	PROM (n=30)		Prolonged labour(n=23)		Controls(n=19)	
	No. of mothers	%	No. of mothers	%	No. of mothers	%
E. Coli	18	60	11	47.8	13	68.4
Staph. Aureus	10	33.3	8	35	11	58
Staph. Epidermidis	10	33.3	10	43.5	6	31.6
Strep. Pneumonia	1	3.3	1	4.3	1	5.3
Strep. Viridans	2	6.6	1	4.3	4	21
Klebsiella	3	10	2	8.6	0	0
N.Gonorrhea	2	6.6	1	4.3	0	0
Antracoides	6	20	2	8.6	4	21
Candida	4	13.3	1	4.3	3	15.8
Diphtheroids	1	3.3	3	13	0	0

This table lists the organisms obtained from the mothers' high vaginal swabs and their frequencies. *E.coli*, *Staph aureus* and *Staph epidermidis*, in that order of frequency, were the most common organisms isolated. All high vaginal swabs resulted in +ve cultures.

Table 12: **Profile of organisms isolated from the conjunctivae of the newborns.**

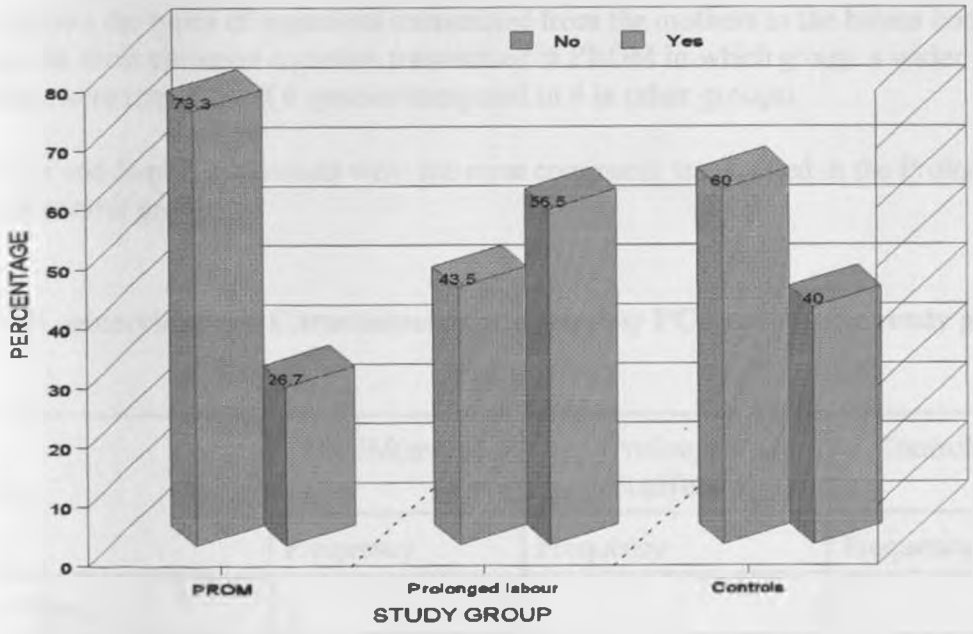
Organism	PROM (n=32)		Prolonged labour(n=34)		Controls(n=28)	
	No. of neonates	%	No. of neonates	%	No. of neonates	%
Staph. epiderm.	12	37.5	13	38	14	50
Staph. aureus	8	25	16	47	11	39
Strep. pneumonia	2	6	2	6	0	0
Strep. viridans	2	6	1	3	3	10.7
E. coli	5	15.6	3	9	1	3.6
Klebsiella	1	3	0	0	0	0
N.gonorrhea	1	3	0	0	0	0
Anthracoides	1	3	0	0	0	0
Candida	1	3	0	0	0	0

This table outlines the type of organisms isolated from the newborns' conjunctiva. *Staph. epidermidis*, *Staph aureus* , *E.coli* and *Streptococcus viridans* were the 4 most common organisms isolated.

Table 13: Transmission of organisms in cases and controls.

Organism the same in mother and baby	PROM		Prolonged labour		Controls	
	Frequency	%	Frequency	%	Frequency	%
No	22	73,3	10	43,5	12	60
Yes	8	26,7	13	56,5	8	40
Total	**30**	**100**	**23**	**100**	**20**	**100**

Figure x: Transmission of organisms in cases and controls.



Transmission rates:
This table/figure shows the rates of mother-baby organism transmission in the 3 study groups. The highest transmission occurred in PL (57%), controls followed with 40% and PROM had 27%. However, the higher transmission in PL did not attain statistical significance (Odds ratio=1.95, P value 0.270).

Table 14: Distribution of transmitted organism in the study group.

Organism	PROM(n=8)	Prolonged labour(n=13)	Controls (n=8)
	No. of pairs	No. of pairs	No. of pairs
Staph. aureus	1	5	4
Staph. epiderm.	1	5	2
E. coli	3	2	1
Strep. viridans	1	1	1
N.gonorrhea	1	0	0
Antracoides	1	0	0

This table shows the types of organisms transmitted from the mothers to the babies conjunctivae. *E. coli* was the most common organism transmitted in PROM in which group a wider spectrum of organisms were transmitted(6 species compared to 4 in other groups).

Staph aureus and *Staph epidermidis* were the most commonly transmitted in the Prolonged Labour and control groups.

Table 15: N. gonorrhea and C.trachomatis as isolated by PCR among the study groups

Organism	PROM(n=16)	Prolonged labour(n=15)	Controls(n=15)
	Frequency	Frequency	Frequency
N. gonorrhoea	3	1	1
C. Trachomatis	1	0	0

This table shows the frequencies of obtaining *N.gonnorrhe*a and *C.trachomatis* from conjunctival swabs by the PCR method. These organisms were obtained more frequently in the PROM group.

Table 16: Resistant patterns for gram positive organisms.

Drugs	*Staph aureus* (n=36)		*Staph epiderm* (n=38)		*S.pneumonia* (n=4)		*S. viridans* (n=6)		*Antrhracc* (n=1)
	R	%	R	%	R	%	R	%	
Tetracycline	28	80	26	68	1	30	2	40	R
Ofloxacin	0	0	0	0	0	0	0	0	S
Gentamycin	7	20	6	16	0	0	6	100	S
Kanamycin	25	70	22	58	N/A		N/A		N/A
Augmentin	0	0	0	0	0	0	0	0	N/A
Ciprofloxacin	0	0	0	0	N/A		N/A		N/A
Penicillin	28	80	28	74	0	0	0	0	S
Cefotaxim	7	20	6	16	0	0	0	0	N/A
Neomycin	0	0	2	5	1	30	5	80	S
Tobramycin	0	0	0	0	N/A	0	N/A		N/A
Imipenem	0	0	0	0	0	0	0	0	S
Erythromycin	7	20	12	32	0	0	0	0	N/A
Vancomycin	N/A		0	0	0	0	N/A		N/A
Chloramphenicol	4	10	6	16	0	0	0	0	S
Clindamycin	4	10	12	32	0	0	0	0	N/A
Cephalexin	14	40	2	5	N/A	0	N/A		N/A
Oxacillin	11	30	2	5	0	0	4	60	N/A
Amoxicillin	18	50	11	29	0	0	0	0	R
Ceftazidin	N/A		0	0	N/A	0	0	0	N/A

This table shows the percentages of organisms resistant to a particular drug.

NA: Not applicable (antibiotics not relevant for a particular organism)

n Total number of isolates tested for the drug

r Number of resistant isolates to a particular antibiotic, R Resistant, S Sensitive

A good effective drug is one with resistance of 10% or less.

Table 17: Resistance pattern for gram negative organisms isolated:

Drugs	*E.Coli* (n=10)		*Klebsiella* (n=1)	*gonorrhea*(n=1)
	r	Percent		
Tetracycline	6	60	S	NA
Ofloxacin	0	0	S	S
Gentamycin	0	0	R	NA
Kanamycin	0	0	NA	NA
Ciprofloxacin	0	0	S	S
Penicillin	8	80	R	R
Cefotaxim	0	0	NA	NA
Neomycin	0	0	S	S
Imipenem	0	0	NA	NA
Erythromycin	10	100	R	S
Polym B.	0	0	NA	NA
Vancomycin	0	0	NA	S
Chloramphenicol	0	0	NA	S
Clindamycin	10	100	R	NA
Cephalexin	0	0	R	S
Oxacillin	0	0	R	R
Amoxicillin	4	40	R	S
Ceftazidin	NA		NA	R

NA: Not applicable (antibiotics not relevant for a particular organism)
n Total number of isolates tested for the drug
r Number of resistant isolates to a particular antibiotic
R Resistant
S Sensitive
A good effective drug is one with resistance of 10% or less.

Table 18: Hierarchy of general drug resistance pattern:

Drugs	%
Penicillin	72
Tetracycline	64
Kanamycin	50
Amoxicillin	38
Oxacillin	31
Erythromycin	24
Clindamycin	23
Gentamycin	22
Cefhalexin	18
Cefotaxim	14
Chloramphenicol	13
Neomycin	9
Augmentin	3
Vancomycin	3
Ofloxacin	1
Ciprofloxacin	0
Tobramycin	0
Imipenem	0
Ceptazidin	0

This table shows the general drug resistance pattern. Kanamycin is notably ranking third.

DISCUSSION

This study was carried out at Kenyatta National Hospital and Pumwani Maternity Hospital, two of the major Nairobi city hospitals undertaking a high number of maternity deliveries. They also serve as referral centers for many other satellite city, and neighbouring district hospitals.

The study looked into 161 mother-baby pairs. 52 of the pairs were from the mothers who had Premature Rupture of Membrane (PROM), 54 had Prolonged Labour(PL) and both groups were controlled with 55 patients who had neither PROM nor PL (Figure i).

Table 2 (Figure ii) shows how the mothers were distributed by age. The mode for the ages was 23 years for the PROM and controls whereas younger patients were seen in the PL group (mode =18). This is expected as the younger, primigravidas are the ones who had difficulties with labour.[13, 18] There was no difference in the means of ages in the three groups (P=0.32).

Most newborns were seen and their conjunctival swabs taken, on average, 24 hours after birth (Table 3). The spread of ages by the time conjunctival swabs were taken was the same for all the three groups (figure iii). Figure (iv) shows that microorganisms colonized the newborn eyes by as early as 12 hours. Interestingly, newborns of PROM patients were colonized more frequently and earlier (60%), compared to 33% in PL and controls. Newborns of PROM and PL groups thence showed a steady rise in the number of positive cultures with increasing age. The controls showed only a minimal rise. As was illustrated by Duerden et al, newborns became colonized within hours of birth.[1] The higher and early colonization in newborns of

PROM, could be explained by the fact that most rupture of membranes were caused by pre-existing and established infectious agents, which colonized the conjunctiva in-utero, and only proliferated more after birth.[15] The agents in PL and controls were acquired during parturition and took some time before becoming established in the conjunctivae of newborns. Culture positivity showed little change with increasing age in the control group, probably due to low initial colonization at the time of delivery. It would have been interesting to see the behaviour of colony counts were it possible to do them.

Table 5 shows, a poor scenario of ocular prophylaxis. There was virtually no prophylaxis for neonatal conjunctivitis given in the two hospitals. The only two cases that received antibiotics were only given after detection of conjunctivitis while still in the ward. This trend had been persisting for over 10 years, when it was last observed and documented. The reasons given for this status were poor availability and affordability of drugs,[31] but on top of that, the authors observed poor motivation of the health workers to give prophylaxis. The need for neonatal prophylaxis seems to be an only ophthalmologists concern with other sectors oblivious of it.

As is also shown in table 5, most children seen were mature (>37 wks). This is in keeping with earlier studies done on the two conditions. In a study done in USA on PROM, 94% of fetuses were mature, and most of the prolonged labours were caused by cephalo-pelvic disproportion, in which cases the fetuses were big and mature.[13, 18] Most of the newborns studied were delivered by spontaneous vaginal delivery. The only one delivered by caesarian section in PROM had a positive ocular culture but did not develop conjunctivitis. None of the others delivered by caesarian section in PL and controls had either positive culture or

developed conjunctivitis. Most mothers were of low parity (mean = 2.0). The number of mothers with prenatal vaginal infection correlated well with those who received antibiotics two weeks before and during delivery. It was observed that PROM had higher proportion of mothers with antenatal vaginosis (15%) compared to 10% and 9% in PL and controls respectively. This, as was seen in the preceding paragraph, could explain why neonates of mothers with PROM were colonized more and earlier than the other two groups.

Table 6 (figure v) shows the distribution of the results of conjunctival cultures by the study groups. In PROM and PL groups, positive cultures were obtained more frequently than negative cultures, 63% (odds ratio = 1.9, P-Value 0.09) for PL and 62% (odds ratio = 1.6, p-value = 0.20) for PROM as compared with the 50% by 50% in controls. In a study done in Kikuyu hospital in 1991-1993 by Isenberg at al, it was found that 55% of patients with conjunctivitis were culture positive.[31] The higher frequency of positive cultures in PROM and PL as shown in this study, though not reaching significant levels, clearly implicated these two as risk factors for conjunctival colonization. As PROM presented a pre- existing infective state, PL on the other hand presented a situation in which the mother was subjected to repeated digital vaginal examinations. These might have exposed both the mother and the infant to microbial contamination.

Incidence rates for neonatal conjunctivitis for the three groups averaged 23% (Table 7-figure vi). The rates of developing conjunctivitis were higher in both the PL (31%) and PROM (27%) than in the controls (23%). Although the differences are not statistically significantly higher than in the controls, clinically PROM and PL stood out as special risk factors. The average rate of 23% found in this study corresponded with that quoted in earlier studies of 1986 by Fransen, Klaus et al and 1991 – 1993 by Isenberg et al [3,17,31] and therefore indicated

that there had been no change in incidence in the preceding 10 years or so. Mothers in the control group were subjected to the routine methods of delivery. They underwent digital vaginal examinations as frequently as it deemed necessary. These kind of unchecked digital examinations could have exposed them to microbial contaminants. As this group represented the usual scenario in the general ward set-up, it revealed that the problem existed even in situations of normal delivery, and therefore emphasized the need for observing infection control in all deliveries. Ocular prophylaxis should be availed to all neonates.

Table 8 (figure vii) shows the relationship between conjunctival cultures and development of conjunctivitis. This relationship proved statistically significant in PROM, in which 86% of neonates with positive cultures developed conjunctivitis, (Odds ratio = 2.73, P-value = 0.037). In the study done in Kikuyu Hospital 1991-93, positive cultures were found in 55% of those with conjunctivitis. [31] The higher proportions obtained in this study compared with the one done at Kikuyu, could be explained by the fact that it looked at PROM, a special risk group of patients, whereas the other looked at a general population. This again implicated PROM as a risk factor for development of conjunctivitis. PL and controls had similar but lower frequencies of conjunctivitis in those with positive cultures than for PROM, which probably indicated that the microbial contaminants involved, though more frequent obtained, were of low virulence and probably colonized conjunctivae less.

Table 9 shows the relationship between the length of PROM (latency periods) and the conjunctival cultures and conjunctivitis. As illustrated in figure 8, the longer the latency, the higher the frequency of obtaining positive cultures. This was in keeping with the trends quoted in literature. [13, 17] Frequently more positive cultures were observed especially above 24

hours of latency. There was no relationship found between latency and development of conjunctivitis. This was not in keeping with other studies, which implied that long latencies led to higher neonatal morbidity. [12, 13] This might have meant that in PROM, the risk for neonatal conjunctivitis was associated more to the infective organism rather than the mere length of broken membranes. Figure viii and table 14 again re-emphasized this point.

A positive correlation was found between the duration of prolonged labour and both positive conjunctival cultures and conjunctivitis (Table 10- figure ix). Long durations of prolonged labour, especially beyond 16 hours, were positively related to higher positive cultures though statistical significant levels were not attained (P=0.139). Likewise, long duration of prolonged labour was significantly related to development of conjunctivitis (P=0.029). This was in keeping with other studies that showed that prolonged labour was related to higher neonatal sepsis. Prolonged duration of labour was related to higher number of per-vaginal examinations, longer exposure of neonatal eyes to vaginal secretions and flora, and due to in many cases of protracted labour, increased facial bruising. All these rendered the neonate susceptible to conjunctival infections.[23]

The study also looked at maternal high vaginal swab cultures, which were virtually all positive. This was expected as the vaginal canals were not sterile and had their resident microbial florae, that proliferated after delivery due to increased blood breakdown products.[1] Table 11 shows the profile of organisms isolated from the high vaginal swabs. *E.coli, staph aureus* and *Staph. epidermidis* were the most common organisms isolated. *E.coli* is an *enterobacteriacia* that contaminates the vaginal parts from the ano-rectal region. *Staphylococci* are skin florae that also contaminate the uro-genital parts.

Table 12 shows the types of organisms isolated from the neonates' conjunctival swabs. In this profile, *Staph epidermidis*, *Staph aureus* and *E. coli* in that order of frequency were the most common isolates. Other isolates included; *Strep. Pneumonia, Strep viridans, Klebsiella, Neisseria gonorrhea, Anthracoids* and *Candida species*. The most common species were the skin florae, *Staphylococci*, which most probably came from the vaginal contaminants during delivery. These species could also have been acquired from the infant's post-delivery immediate environment.[17] The other species like *N.gonorrhoea* and *S.pneumonia* are true pathogens in the vaginal flora and were isolated more frequently in the mothers with PROM.

In a sub-group of 46 neonates PCR was done for *N. gonorrhea* and *C. trachomatis*. *Neisseria gonorrhea* was isolated in three patients out of 16 in PROM group, one out of 15 in PL group and one out of 15 in control group. *Chlamydia* was isolated in only one out of the 16 patients in the PROM group (table 15). These figures indicated that *N. gonorrhea* was still more of a problem than *C.trachomatis*. [5, 6] Overall *N.gonorrhoea* was isolated in 11% of the neonates and *C. trachomatis* in 2.2%. These figures, which were much lower than those quoted in the 1986 study by Klaus et al (24%) and by Isenberg et al 1991- 1993 (25%-50%), [3, 31] may be indicative of a downward shift of the role *N. gonorrhoea* and *C. trachomatis* are playing in the causation of neonatal conjunctivitis, and probably taking second position after other microbial organisms. PCR on HVS from mothers of neonates with Chlamydial and gonoccocal conjunctivitis could not be done due to cost constraints. It would have been ideal to determine whether the *Chlamydia* and *gonorrhea* came from the mother and the rates of isolation of these organisms from the mothers of the infants with conjunctivitis.

Table 13 (figure x) shows the frequency of obtaining the same organism in a mother-baby pair. Assuming that, if the same organism was found in both the baby and the mother, that it came from the mother, and that such was a positive transmission, an overall transmission rate of 40% was found. Subjects in the PL group had the highest transmission rate of 57%, controls 40% and PROM group 27%. Transmission in the PL group was much higher than in the control group (odds ratio= 1.95) though statistical significance was not reached (p=0.27). These rates were similar to the ones quoted for *chlamydia* (20–50%) and for *N. gonorrhea* (30-50%) by the American sexually transmitted disease information center of 1996.[32] Patients with PROM enjoyed relative protection from undue contamination by repeated vaginal examinations. In these patients digital vaginal examinations are prohibited and the number of speculum examinations limited. In some instances intravenous antibiotics would be given in the intravenous syntocinon drip, during labour induction. If such a drug escaped indication in the treatment sheet, it was considered as 'no antibiotic use'. This would have influenced the culture results and hence the transmission rates obtained. As expected and as previously shown, long duration of labour exposed the neonate's eyes to vaginal flora hence the high transmission rates. The control patients, who delivered within 12hrs, probably received no antibiotics during labour and the number of vaginal examinations was unchecked, thus producing higher transmission rate than that of PROM though not as high as of PL. This was the scenario in the general set-up, which raised concern over the level of protection the newborns delivered uneventfully received.

Table 14 shows that the types of organisms transmitted in PROM group were different from those transmitted in PL and control groups. *E.coli* was the most common organism transmitted in the PROM group whereas *Staph epidermidis* and *Staph aureus* were the most

common in the other two groups. Other organisms transmitted in relatively higher proportions in the PROM group than in the other study groups were; *Anthracoids, N. gonorrhea* and *Strep. viridans*. These probably would explain two observations: Firstly, that the higher rate of conjunctivitis in those with positive ocular cultures in PROM group might have been because of more virulent organisms being involved and transmitted to the eyes of the newborns, other than the mere fact of having broken membranes. Notably *N.gonorrhoea* and *Strep.viridans* were significantly associated with conjunctivitis (P = 0.06, P = 0.017 respectively). Secondly, that the organisms obtained in the PL and control groups were those known to be contaminants from skin. Repeated digital vaginal examinations could explain the source of contamination of such maternal vaginal canals and subsequent transmission to the newborns' eyes. [23] These organisms were probably of low virulence and therefore probably the reason for poor association with development of conjunctivitis.

Table 16 and 17 show drug resistance patterns for the gram positive and gram-negative organisms respectively. Both groups of organisms showed multiple drug resistance especially to the commonly used drugs in these hospitals.

Staph.aureus was found to be resistant to Tetracycline, Kanamycin, Penicillin and Amoxicillin, but susceptible to Aminoglycosides, Flouroquinolones and Cephalosporines. *Staph epidermidis* showed resistance to Tetracycline, Kanamycin and Penicillin and susceptible to Flouroquinolones, Cephalosporines, Aminoglycosides and Cephalosporines.

Strep pneumonia showed susceptibility to all antibiotics tested. This was different from that shown in a study by Dr. Patel A, in children who had nasolacrimal duct obstruction, in which

Strep pneumonia showed resistance to Tetracycline, Erythromycin and Ampicillin.[33] *Strep. viridans* showed resistance to Gentamycin, Neomycin and Oxacillin. It was susceptible to Cephalosporines, Flouroquinolones, Clindamycin, Vancomycin and Chloramphenicol.

E.coli showed resistance to Tetracycline, Penicillins, Erythromycins and Clindamycin, but was susceptible to Aminoglycosides, Flouroquinolones and Cephalosporines.

Klebsiella showed resistance to Gentamycin, Penicillins, Erythromycins, Clindamycin, Cephalexin, Oxacillin and Amoxicillin but susceptible to Flouroquinolones.

N. gonorrhea showed resistance to Penicillins, Oxacillins and Ceftazidime. It was susceptible to Flouroquinolones, most Cephalosporines and Aminoglycosides.

Table 18 shows the general pattern of resistance. Penicillin, Tetracycline and Kanamycin had resistance of 50% and more. The most effective drugs were Flouroquinolones, Augmentin, Tobramycin, Neomycin and Vancomycin with resistance of 10% and less. Kanamycin, a drug that has been shown to be effective in earlier studies, ranks number three in those with high resistance in this study. Probably resistance is developing to it as it becomes more available for the treatment of sexually transmitted diseases.

Information on the pattern of bacteria species and their antibiotic susceptibility help to improve on patients' care where clinical diagnosis only may be used to initiate treatment. It saves on time to initiate such treatment especially where laboratory facilities are not

accessible or affordable. Such patterns should be determined for different geographical regions and from time to time, as they are bound to change.

Conjunctival cultures from neonates of PROM patients grew *E. coli* in high frequencies. Cheap drugs that it was shown to be sensitive to included Neomycin, Chloramphenicol, and Gentamycin. Ciprofloxacin, Ofloxacin and Imipenem showed higher sensitivity.

Neonates of mothers with PL and normal deliveries grew *staphylococcal* species in high frequencies. They were found to be sensitive to the following antibiotics: Neomycin and Tobramycin. Ciprofloxacin, Ofloxacin, Augmentin, Vancomycin, and Imipenem showed superior sensitivity.

CONCLUSIONS

1. PROM and PL increased the risk of transmission of maternal vaginal flora to the newborn eyes.

2. The rate of transmission of maternal vaginal flora to the newborn eyes was 57% in the group with Prolonged Labour, and 27% in those with PROM, compared to 40%in
   controls.
3. The rates of developing conjunctivitis were higher in PROM (27%) and PL (31%) than in controls 23%. Overall incidence rate was 23%.

4. The longer the duration of Prolonged Labour, the higher was the frequency of obtaining positive ocular cultures and the higher the proportion that developed neonatal conjunctivitis.

5. A positive conjunctival culture in a newborn of a mother with PROM implied that the newborn would most likely develop conjunctivitis.

6. *Staph aureus, Staph epidermidis* and *E-coli* were the most common organisms isolated from the eyes of newborns in all the three groups.

7. Whereas *Staph.* Species were the most commonly transmitted organisms in PL and control groups, *E-coli* was the most common in PROM.

8. Most organisms showed resistance to Tetracycline, Kanamycin and Penicillins. The most effective drugs were Flouroquinolones, Augmentin, Tobramycin, Neomycin, Imipenem, Vancomycin and Ceftazidin.

RECOMMENDATIONS

1. All newborns should receive prophylaxis for neonatal conjunctivitis. A campaign to sensitize all sectors and levels of heath care on the need for ocular prophylaxis should be carried out.
2. All infants born of mothers with PROM should be subjected to conjunctival culture and sensitivity, and treated appropriately.
3. Newborns of mothers with Prolonged Labour should be given antibiotic prophylaxis and actively followed up in the first month of life.
4. In the absence of laboratory culture facilities, the authors recommend the following:

   Newborns of mothers with PROM should be treated with Neomycin, Chloramphenicol, or Gentamycin and reviewed in a week. Where available, Ciprofloxacin, Ofloxacin Vancomycin or Imipenem would be better bargains.

   Newborns of mothers with PL should receive Neomycin, or Tobramycin once and then followed up at least once in the month. Ofloxacin or ciprofloxacin would be ideal where available.

   Newborns born uneventfully should likewise receive neomycin, or Tobramycin once and told to return prn. Ofloxacin or Ciprofloxacin would again be ideal where available

ACKNOWLEDGEMENT

1. To my supervisors Drs. Ilako and Masinde for their guidance and support.
2. To the staff of microbiology, especially Mr. Onduko, Mr. Onyono Mr. Juma and the late Mr. G. Maitha for their technical support.
3. To Dr. Craig Cohen, of microbiology Annex for giving me access to the PCR facilities.
4. To Dr. Nelly Mugo for her advice especially on epidemiological and obstetrical aspects of the study.
5. To Mr. Njeru, Director of Clinical Epidemiology Unit (INCLEN), for his advice and guidance on appropriateness and use of statistical tools.
6. To Ms. Janet Musia for her assistance with data processing and analysis.
7. To Mr. Njani Nderitu, of Eye Modes Nairobi, for letting me have unlimited access to his computer material.
8. To Charity Ndirangu of Eye Modes Nairobi, for her typing services.
9. To my family, for their unrelenting patience and support.

APPENDIX I

ANTIBIOTIC TESTING

Antibiotics for gram positive organisms

Gentamycin (GM), Tobramycin (NN), Neomycin (N), Kanamycin (K), Erythromycin (E), Ofloxacin (OFX), Norfloxacin (NOR), Ciprofloxacin (CIP), Cefotaxime (CTX), Cefalexin (CN), Chloramphenicol (C), Tetracyclin (TE), Ampicillin (AM), Vancomycin (VA), Imipenem (IPM), Methicillin (M).

Antibiotics for gram negative organisms

Gentamycin (GM), Tobramycin (NN), Neomycin (N), Kanamycin (K), Ofloxacin (OFX), Norfloxacin (NOR), Ciprofloxacin (CIP), Cefotaxime (CTX), Cefalexin (CN), Chloramphenicol(C), Ampicillin (AM), Imipenem (IPM), Ceftazidin (CAX), Ceftriaxone (CF), Colistin (CL), Polymyxin B (B).

APPENDIX II

QUESTIONNAIRE

Maternal profile

1. Date____/____/_____ Res. Number_____KNH/PMH

2. Ip.number _____ case _____ control _____

3. Name of mother _____ age (yr.)_____ parity_____

4. Duration of labour prolonged (>12 hrs) yes_____no_____

   If yes a) onset of labour date _____time_____

   B) End of 2nd stage date _____time_____

   Total time (b-a) hrs _____

5. Premature rupture of membranes: yes_____ no_____

   If yes a) time of rupture date _____ time_____

   b) time of onset of labour date _____ time_____

   Total time (b-a)hrs _____

6. Antibiotic (s) to mother within 2 weeks to delivery yes _____ no _____

   if yes specify _____

7. Antibiotic (s) to mother during & after deliver yes_____no_____

   If yes specify _____

8. History of antenatal vaginal discharge? Yes_____no_____

   Or

9. Chorioamnionitis? Yes_____no_____

10. Date swab taken_____ time_____

Newborn profile

1. Name of newborn _____ Res. No _____
2. Sex: M __ _____ F_____
3. Gestation age (at delivery) Term (>37 wk) _____preterm (<37wks) _____
4. Date swab taken _____/_____/_____ time _____
5. Date delivered _____/_____/_____ time_____

   Age(hrs) _____
6. Mode of delivery: svd _____ cs_____ assisted_____

   Presenting part (specify) _____
7. Ocular medications prior to taking swabs yes _____no_____

   if yes specify _____
8. Ocular signs of conjunctivitis? Yes_____ no_____

   if yes specify;

   Lid edema/swelling _____

   Conjunctival injection _____

   Conjunctival chemosis _____

   Mucoid discharge _____

   Waterly discharge _____

   Purulent discharge _____

   Others (specify) _____

Laboratory work-up

1. Date_____ Res.no. _____KNH/PMH

2. Vaginal smears: organism (s) seen at microscopy

   (i)

   (ii)

3. Vaginal swabs: culture positive_____ negative_____

   a) If positive, organisms isolated

   (i)

   (ii)

   b) Vaginal culture; sensitivity pattern _____ _____

   _____

4. Ocular smears: organism (s) seen at microscopy

   (i)

   (ii)

5. Ocular swabs: culture: positive _____ negative_____

   If positive

   Organism isolated colony counts

	Light (<20)	moderate (>20-100)	heavy (<100)
(i)	_____	_____	_____
(ii)	_____	_____	_____

6. Ocular organisms: sensitivity pattern _____

_____

_____

Follow up visit at MCH

Date_____ Res. Number_____

lp.number_____ case_____ control_____

Name of infant _____ age (in weeks) _____

Ocular signs of conjunctivitis? Yes_____ no _____

if yes, indicate which

Lid oedema _____

Conjunctival injection _____

Conjunctival chemosis _____

Mucoid discharge _____ _____

Waterly discharge _____

Purulent discharge _____

Others (specify) _____

Organisms seen at microscopic smears

i)

ii)

Culture; positive_____negative_____

If positive, organisms isolated

i)

ii)

REFERENCES

1. Duerden, Reid, Turk: A new textbook of microbial and parasitic infections: 29.
2. Kanski: Clinical ophthalmology; 3rd ed, 83
3. Alexander AB, Klaus PS: Neonatal ophthalmia in tropical countries: Tropical infectious diseases of the eye: 646.
4. O'Hara M.A: Ophthalmia Neonatorum: Paed. Clinic of N. America; Aug. 1993 40 3: 715-25.
5. Franzen L, Nsanze H, Klaus V, et al: Ophthalmia Neonatorum in Nairobi Kenya, The role of C. trachomatis and N. gonorrhea. Journ. Of Infectious diseases; (1986): 75
6. Laga M, Plummer F, Nsanze H, et al: Epidemiology of Ophthalmia Neonatorum in Kenya: 1986, Lancet 2:1145-48.
7. Nsanze H, Dawodu A, Lismani A, et al: Ophthalmia Neonatorum in United States Emirates: Ann Tropical Pediatrics March 1996; 16 1: 27-32.
8. Isenberg SJ, Apt L, Wood M: The influence of perinatal infective factors on Ophthamia Neonatorum: Journal. Of Paed. Ophthalmology and strabismus. May – June 1996; 33 3: 185-88.
9. Dalta P, Masinde S, Wamola I et al: Ophthalmia Neonatorum in a trachoma endemic area: Journ. Sexually transmitted diseases (United States) Jan-Feb.1994; 21 1:1-4
10. Bartlett, Jaanus: Clinical Ocular Pharmacology: 652-654
11. Mani VR, Vidya KC: A microbial study of Ophthalmia Neonatorum in hospital born babies: Journ. Indian Medical association (INDIA) July 1997; 95 7: 416-17.
12. Steinfield JD, Lenkoski C, et al: Neonatal morbidity at 34-37 weeks, the role of ruptured membranes: Obs. Gynecology July 1999; 94 1:120-3.

13. Parnoll, Benson: Current Obstetrics and Gynaecological Diagnosis and Treatment: 7th Ed: 332, 496.

14. Halla K, Bolta S.F, et al: Fetal Diagnosis and Therapy: may-June 1999; 14 3:138-42.

15. Mikamo H, SatoT, et al: Bacterial isolates from patients with preterm labour with and without preterm rupture of fetal membranes: Infect. Disease Obs. Gynaecology 1999; 7 4: 190-4.

16. Lewis H, Welson, et al: Expectant management of Preterm premature rupture of membranes: American Journal of Obs.Gynaecology Aug. 1994; 171 2: 1162

17. Roy MP, James RS: Premature rupture of membranes: clinical Obs/Gynaecol, Dec.1991; 41 4. 75

18. Jos Van Roosemalen MD: Maternal height and prolonged labour in rural Tanzania: Journal of Obs. Gynaecol. Of Eastern and Central Africa; (1991) 9 2. 1234

19. Denise de Andrade et al: Journal of public health April 2000; 34 2. 506

20. Vaughan Asbury: General Ophthalmology: Lange 1980; 10th Edition: 331.

21. Peter GB. Johnson: The newborn child; (1994): 126.

22. Centers for Disease Control and Prevention. Recommendations for the prevention and management of Chlamydia trachomatis infections, 1993: MMWR 1993; 42(RR-12): 1-39.

23. Kihara AB: To determine the patterns of early perinatal morbidity/mortality associated with augmentation of labour with oxytocin in Nyeri P.G.H: unpublished M.Med. Thesis 1999.

24. De Toledo AR, Chandler JW: Conjunctivitis of the newborn: Infectious disease clinic of North America (United States) Dec. 1992; 6 4: 807-13.

25. Danning L, Stravne B, et al: Ophthalmia neonatorum in northern Norway, microbiology with emphasis on chlamydia trachomatis: Acta. Ophthalmol. (Copenhagen) (Denmark) Feb 1992; 70 1: 19-25.

26. Fransen L. Nsanze H. Klaus V. et al: Ophthalmia Neonatorum in Nairobi, Kenya. The role of Neisseria gonorrhea and C. trachomatis: Journ. Of Infectious diseases; (1986): 153.

27. Orwenyo EA: A study of cervical infections with N. gonorrhoea, C. trachomatis and Herpes simplex Virus during 3rd trimester of pregnancy at KNH: unpublished M.Med. Thesis 1984.

28. Van Bogaert LJ: Ophthalmia neonatorum revisited: Afr. Journ. Of Reprod. Health (Nigeria) April 1998; 2 1; 81-6.

29. Remir K, Fleisher W, et al: Povidone – Iodine liposomes- an overview: Dermatology (Switzerland) 1997; 195 Suppl. 2: 93-9.

30. Isenberg SJ, Apt L, Yoshimori R, et al: Povidone iodine for Ophthalmia neonatorum prophylaxis: Ann Journ. Ophthalmol. (United States) Dec.1994; 118 6: 701-6.

31 Sherwin J, Isenberg, Leonard Apt, M. Wood: A controlled trial of povidone iodine as prophylaxis against ophthalmia neonatorum: New England Journ. Of Medicine 332; 562-566 (march 2), 1995

32. American Sexually Transmitted Disease Center: Guide To Clinical Services: 2nd Ed; U.S. preventive Task Force (USPSTF), 1996

33. Patel Aditi MD: Congenital Nasolacrimal Duct Obstruction: prevalence, bacteriology, and management as seen in Nairobi: unpublished M.Med. Thesis 1999.

34. Bauer, Kirby, Sherris and Turck 1966: Am. Journ.clinic. Pathol. 45:493