# STUDY OF BACTERIAL FLORA IN THE INITIAL OROPHARYNGEAL ASPIRATE AND BLOOD OF POTENTIALLY SEPTIC NEONATES

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## ABSTRACT

There are a considerable number of admissions of neonates because of vertical transmission of infection. The sterility of amniotic fluid is questionable in cases of premature rupture of membrane (PROM), spontaneous preterm labor, and infection. A Prospective Risk Analytical Study was conducted to evaluate the use of oropharyngeal aspirate in the diagnosis of sepsis in potentially septic neonates born at OB Admitting Section, Philippine General Hospital from March to August 2004. Included were 107 neonates, of these, 15 (14.02%) have maternal risk factor of PROM; 34 (31.78%) have maternal risk factor of spontaneous preterm labor alone; 38 (35.51%) have maternal risk factor of UTI; 15 (14.02%) have maternal risk factor of respiratory tract infection; and 2 (4.6%) have maternal risk factor of fever alone. Immediately after birth, fluid from the oropharyngeal are and blood was sent for culture. The neonates were followed up within 72 hours, and it was noted that 50 (46.73%) developed jaundice, 17 (15.89%) had pneumonia, 7 (6.54%) had necrotizing enterocolitis (stage IA to IV) and 3 (2.80%) had decreased activity, temperature instability and hypoglycemia, collectively grouped under sepsis. Of the 107 subjects, 33.6% have oropharyngeal aspirate growth and 8.4% have blood culture growth. Among the subjects who have oropharyngeal aspirate growth, 25% have a concomitant blood CS growth, irregardless of the organism isolated. Of the subjects who had growth on their blood culture, 77% have the same organism isolated in their oropharyngeal aspirate. PROM and UTI highly correlated with positive growth in the oropharyngeal aspirate. *Acinetobacter baumanii* and *Alkaligenes faecalis* isolated from oropharyngeal aspirate has 100% correlation with blood culture and *E. coli* isolated from the oropharyngeal aspirate correlated 83.33% with blood culture. The sensitivity of oropharyngeal aspirate culture growth irregardless of the organism isolated is 100% and the specificity is 75%. In conclusion, oropharyngeal aspirate when positive for growth may not reflect growth in blood culture, and when negative for growth highly correlates with a blood culture that has no growth.

## INTRODUCTION

One of the biggest goals of doctors in every institution is to decrease the incidence of neonatal septicemia. In the PGH alone, the statistics points unfavorably to the high number of neonatal deaths due to infection. Early detection by surveillance is a must to eliminate high mortality rates caused by infection.

Neonatal mortality due to neonatal infection brought about by maternal infection is being faced hand in hand by pediatricians and obstetricians. Subclinical ascending infections through the lower female genital tract are predominant worldwide. The importance of prenatal check-up has been emphasized, as well as proper hygiene. But still, there are a considerable number of admissions of neonates in the Neonatal Intensive Care Unit because of vertical transmission of infection. Usual source of bacterial infection are the colonizers. The bacteria load may increase and thus, may predispose the immunologically immature newborn to sepsis. The overall mortality rate of neonates with congenital neonatal sepsis ranges between 25 to 90%.

The amniotic fluid is normally sterile, but its sterility is questionable in cases of premature rupture of membrane, spontaneous preterm labor, and infection, especially genitourinary infection and respiratory tract infection.

Preterm labor occurs in 20% of pregnancies in low-income countries whereas prelabor rupture of membranes and septicemia may occur in 5-10% of such settings. A wide variety of bacteria present in the normal vaginal flora of pregnant women such as anaerobes and *Escherichia coli* can also cause ascending infections, usually after rupture of membranes, resulting in intraamniotic infection. Chorioamnionitis resulting from such infections can lead to preterm labor and fetal

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morbidity and in the newborn, were important causes of septicemia and meningitis.

Considerable evidence derived from animal studies shows administration of bacteria in pregnant animals can induce preterm labor and subsequent neonatal morbidity or mortality. Systemic maternal infections have also been associated with preterm labor and neonatal complications. The rate of preterm delivery associated with pneumonia ranges from 15-48%. The Gold standard for diagnosing intrauterine infection is a positive culture of amniotic fluid collected aseptically via transabdominal amniocentesis which that is invasive. Microorganism gain access to the amniotic cavity by ascending from vagina and cervix, hematogenous dissemination (transplacental), retrograde seeding from peritoneal cavity through the fallopian tube, and accidental introduction at the time of invasive procedure. Of these mentioned, the most common pathway is the ascending route of infection. Studies have shown that in virtually all cases of congenital pneumonia, inflammation of chorioamniotic membrane is present. Other studies also show that bacteria identified in cases of congenital infection are similar to the bacteria isolated from the lower genital tract.

Intraamniotic infection is difficult to diagnose. Clinical criteria includes maternal fever, maternal or fetal tachycardia. The infection may be polymicrobial, but collecting amniotic fluid samples without contamination of normal vaginal flora requires invasive procedure. A number of studies have shown a correlation between vaginal infections and preterm birth, The most advanced and serious stage of ascending infection is fetal infection.

The nose and the external ear canal in the newborn infants have bacterial colonizers and in one study done among infants < 7 days old, they noted that the newborn's nose has this pattern of bacterial colonization: normal flora (39%); potentially pathologic gram-positive microorganisms (23%); gram-negative enteric rods (16%); and sterile cultures (22%). The most frequent bacterial isolates from nostrils were *Staphylococcus epidermidis* (39%); *Staphylococcus. aureus* (11%) and *Escherichia coli* (8%). The cultures from the external ear canal showed normal flora (37%); potentially pathologic gram-positive microorganisms (5%); gram-negative enteric rods (24%); and sterile cultures (34%). The most frequent bacteria cultured from the ear canal were: *Staphylococcus. epidermidis* (37%); *E. coli* (8%); and *Klebsiella pneumoniae* (7.5%).[1]

In another study, Gram positive septicemia the commonest site for colonization of the same organism in newborn was found to be umbilicus followed by nose, throat and external auditory canal, while in Gram negative septicemia rectum and umbilicus were the commonest sites.[2]

Oropharyngeal fluid aspirates have not yet been explored yet. In utero, the fetus swallows amniotic fluid. The aspirate that is present in the newborns oropharyngeal area may represent the environmental milieu that the newborn is exposed to while in utero. Swallowing potentially septic amniotic fluid may predispose the newborn to sepsis. Thus, will an initial oropharyngeal fluid aspirate culture reflect the blood culture of potentially septic neonates?

**DEFINITION OF TERMS**

Potentially septic neonates included in the study are those that have the maternal risk factors of infection that occurred in the last trimester of pregnancy. This includes maternal fever alone, maternal urinary tract infection (dysuria, vaginal discharge), maternal respiratory tract infection, prelabor rupture of membranes, and spontaneous preterm labor. Maternal fever as a risk factor is defined in this paper as the presence of temperature of more than 37.5 ° C with no other symptoms and signs pertaining to a focus of infection. Prelabor rupture of membranes denotes spontaneous rupture of fetal membranes before the onset of labor, whether term of preterm. Pregnancies with intact fetal membranes and spontaneous preterm labor must be distinguished for clinical as well as for research purposes from those in which there has been rupture of membranes. Preterm labor is precisely defined as spontaneous onset of labor occurring before the 37 completed weeks or 259 completed days of gestation, without any other symptoms and with intact fetal membranes. If the pregnant mother was having symptoms of dysuria with fever, and had a midstream, clean-catch urinalysis showing elevated Urine WBC of more than 5 per high power field and/or urine culture with more than 100,000 colony-forming units of a single organism per 1mL of urine then she was considered as having maternal urinary tract infection. The presence of cough and/or colds with associated fever was categorized as having respiratory tract infection.

Neonates were followed up and their morbidities that occurred within 72 hours were noted. Jaundice for the purpose of this study was defined as having yellow skin discoloration with an elevated total bilirubin levels increasing >5mg/cL per day, >12 mg/dL in a term infant and >15mg/dL in a preterm infant, direct bilirubinemia >2mg/dL and the jaundice lasted for more than 1 week. Infants categorized as having jaundice had jaundice alone as their main symptom with no other associated symptoms of disease Those that were labeled as having

pneumonia were neonates that developed respiratory distress, rales on lung fields and abnormal temperature patterns. Necrotizing enterocolitis was defined as feeding intolerances (residuals, abdominal distention, vomiting) that were associated with plain abdominal studies supporting the diagnosis (presence of ileus, pneumatosis intestinal or pneumoperitoneum). The neonate was labeled as having sepsis if the neonate had a blood culture isolate and had clinical signs and symptoms compatible with sepsis like hypothermia, hyperthermia, poor suck, lethargic.

The use of initial oropharyngeal fluid aspirate as an alternate means to reflect the possible isolate in the neonate is thought of because the fluid in that anatomical body part reflects the intrauterine environment that the neonate was exposed to. This aspirate was the first isolate that we usually obtain in the newborn because suctioning of the oropharyngeal area was part of initial resuscitation. And because the collection of the specimen is done within minutes from birth, it is not much affected by external, internal and temporal factors.

Only symptoms that developed within 72 hours of life were noted since symptoms after 72 hours of life were more of acquired than congenital. Presence of growth in the blood culture was the gold standard in determining infection.

## OBJECTIVES

This study aims to identify bacterial isolates in the oropharyngeal fluid of potentially septic neonates delivered from mothers with infection risks. The initial blood culture will be correlated with the oropharyngeal fluid isolate. This study aims to identify if the initial oropharyngeal fluid aspirate correlates with the causative organism causing sepsis in the neonate. If this has a relationship then, the use of a more non-invasive and less expensive laboratory examination as an alternate is an option.

This study aims to see if development of symptoms of infection within 72 hours of life correlates with the presence of growth in the oropharyngeal fluid aspirate. Symptoms developing immediately within 72 hours of birth and the result of oropharyngeal fluid aspirate may predict the result of the blood culture.

## METHODOLOGY

Neonates admitted via vaginal delivery in the OBAS, PGH were be selected. Patients that were excluded were those who had no maternal risk for infection and those born via caesarian section. Those included in the study were patients whose mothers had a high risk of amniotic fluid contamination like maternal infection in the last trimester, prelabor rupture of membrane or preterm labor.

The sample size was computed assuming maximum variability with a margin of error of 0.1. The sample size needed for the study is 96 subjects.

A protocol was submitted to the adviser and a copy was sent to Research Information and Development Office for ethical review and for registration.

Informed consent was obtained prior to the delivery of the newborn subject. The fluid in the oropharyngeal area was obtained observing the aseptic technique. A syringe was attached to a suction tip to aspirate the oropharyngeal fluid before the newborn was suctioned using high negative pressure wall suction. The specimens were placed in a sterile vial with 1cc of sterile NSS and was sent to the Microbiology Laboratory for bacterial studies. Concommitantly, a baseline blood culture was extracted, using only 1 site and 1 bottle. The specimen was sent to the Microbiology Laboratory for culture.

Factors that were considered and noted during the neonate's hospital stay during the first 72 hours of life were the development of illness such as jaundice, necrotizing enterocolitis, pneumonia and sepsis.

The study design is a prospective risk analytical study. The difference between the presence or absence of morbidities and maternal risk factors were compared using independent samples t-test.

## RESULTS

A total of 107 subjects fit the inclusion criteria. Table I below shows the maternal risk factors of babies who were included in the study

**Table I- Maternal Risk Factors**

| Maternal risk Factors | Frequency | Percent |
|---|---|---|
| Prelabor Rupture of Membranes | 15 | 14.02 |
| Spontaneous Preterm Labor | 34 | 31.78 |
| Urinary Tract Infection | 38 | 35.51 |
| Respiratory Tract Infection | 15 | 14.02 |
| Fever alone | 2 | 4.67 |
| **Total** | **107** | **100.00** |

The neonates were followed up within 72 hours and the table II shows the morbidities that occurred.

**Table II- Neonatal Morbidities**

| Neonatal Morbidities | Frequency | Percent | Cum |
|---|---|---|---|
| None | 30 | 28.04 | 28.04 |
| Jaundice | 50 | 46.73 | 74.77 |
| Pneumonia | 17 | 15.89 | 90.65 |
| Necrotizing enterocolitis | 7 | 6.54 | 97.20 |
| Sepsis | 3 | 2.80 | 100.00 |
| **Total** | **107** | **100.00** | |

Of the 107 subjects, 33.6% had oropharyngeal aspirate growth and 8.4% had blood culture growth. Among the subjects who have (+) oropharyngeal aspirate growth, 25% have a concomitant blood CS growth, irregardless of the organism isolated. Of the subjects who had growth on their blood culture, 77% had the same organism isolated in their oropharyngeal aspirate.

**Table III- Oropharyngeal aspirate growth**

| Oropharyngeal aspirate | Frequency | Percent |
|---|---|---|
| None | 71 | 66.36 |
| *Bacillus sp* | 2 | 1.87 |
| *Candida* | 2 | 1.87 |
| *Escherichia coli* | 6 | 5.61 |
| *Klebsiella pneumonia* | 2 | 1.87 |
| *Klebsiella rhinoscleromatis* | 2 | 1.87 |
| *Staphylococcus aureus* | 2 | 1.87 |
| *Streptococcus epidermidis* | 12 | 11.21 |
| *Streptococcus viridans* | 3 | 2.8 |
| *Acinetobacter baumanii* | 2 | 1.87 |
| *Alkaligenes faecalis* | 2 | 1.87 |
| *Enterobacter claocae* | 1 | 0.93 |
| **Total** | **107** | **100.00** |

**Table IV- Blood culture growth**

| Blood Culture | Frequency | Percent |
|---|---|---|
| None | 98 | 91.59 |
| *Escherichia coli* | 5 | 4.67 |
| *Streptococcus epidermidis* | 1 | 0.93 |
| *Acinetobacter baumanii* | 1 | 0.93 |
| *Alkaligenes faecalis* | 2 | 1.87 |
| **Total** | **107** | **100.00** |

**Table V- Sensitivity and specificity of Oropharyngeal aspirate culture as plotted against Blood Culture**

| Test | Blood CS (+) | Blood CS (-) | Total |
|---|---|---|---|
| Oropharyngeal aspirate culture positive | 9 | 27 | 36 |
| Oropharyngeal aspirate culture negative | 0 | 71 | 71 |
| **Total** | **9** | **98** | **107** |

Sensitivity: 1.0000 (0.6637, 0.9910)
Specificity: 0.7245 (0.6237, 0.8074)
Predictive Value Positive: 0.2500
Predictive Value Negative: 1.0000

| | Lower bound | Upper bound |
|---|---|---|
| Likelihood Ratio (+): 3.6296 | 2.1628 | 4.8900 |
| Likelihood Ratio (-): 0.0000 | 0.0080 | 0.5160 |

**Table VI- Maternal Risk Factor Contributing to Presence of Oropharyngeal aspirate Growth**

| Maternal Risk Factor | Oropharyngeal aspirate | | Total |
|---|---|---|---|
| | No growth(%) | With growth | |
| Prelabor Rupture of Membranes | 3<br>20% | 12<br>80% | 15 |
| Preterm Labor | 27<br>79.41% | 7<br>20.59% | 34 |
| Urinary Tract Infection | 26<br>68.42% | 12<br>31.58% | 38 |
| Respiratory Tract Infection | 12<br>80% | 3<br>20% | 15 |
| Fever | 3<br>60% | 2<br>40% | 5 |
| Total | 71<br>66.36% | 36<br>33.64% | 107 |

Fisher's exact = 0.001

There was a significant difference among the maternal risk factors with regards to presence of oropharyngeal aspirate growth. Of all the maternal risk factors, prelabor rupture of membrane has the highest positive to negative ratio of oropharyngeal aspirate growth.

**Table VII- Neonatal jaundice**

| Test | Blood CS (-) | Blood CS (+) | Total |
|---|---|---|---|
| Oropharyngeal fluid aspirate (-) | 33 | 0 | 33 |
| Oropharyngeal fluid aspirate (+) | 17 | 0 | 17 |
| **Total** | **50** | **0** | **50** |

**Table VIII- Neonatal pneumonia**

| Test | Blood CS (-) | Blood CS (+) | Total |
|---|---|---|---|
| Oropharyngeal fluid aspirate (-) | 8 | 0 | 8 |
| Oropharyngeal fluid aspirate (+) | 3 | 6 | 9 |
| **Total** | **11** | **6** | **17** |

Sensitivity : 1.0000 (0.5407,0.9864)
Specificity : 0.7273 (0.3952,0.9256)
Predictive value positive : 0.6667
Predictive value negative : 1.0000

| | | Lower Bound | Upper Bound |
|---|---|---|---|
| Likelihood Ratio (+): | 3.6667 | 1.3918 | 9.6124 |
| Likelihood Ratio (-): | 0.0000 | 0.0119 | 0.7335 |

**Table IX- No symptoms**

| Test | Blood CS (-) | Blood CS (+) | Total |
|---|---|---|---|
| Oropharyngeal fluid aspirate (-) | 26 | 0 | 26 |
| Oropharyngeal fluid aspirate (+) | 4 | 0 | 4 |
| **Total** | **30** | **0** | **30** |

## DISCUSSION

The noted limitation of this study is a small sample size with a wide margin of error, making wide confidence interval and thus compromising the precision. This study has some biases, as not all mothers with risk factors, who have potentially septic neonates, were recruited. The recruitment of subjects and collection of specimen is according to the time that is convenient to the investigator. Confounding factor identified in the study is the administration of antibiotics to the mother. To delimit, larger population must be included, ideally all who have risk factors. However, the confounding factor of antibiotic use cannot be controlled as it would be unethical to defer administration of antibiotics if the clinical suspicion of infection is likely.

The most common maternal risk factor of neonates noted is urinary tract infection, however, majority of them have no oropharyngeal fluid bacterial growth. There are only fifteen (15) neonates with maternal risk factor of prelabor rupture of membrane, but it can be noted that 80% of them have oropharyngeal bacterial growth. The highest blood:oropharyngeal aspirate bacterial growth ratio is the *Escherichia coli.* Cross-examination of variables in Tables VII to IX will show that among neonates who developed jaundice had no bacterial growth in their blood however 34% of them have oropharyngeal fluid aspirate growth. It is important to note that in table VIII, where neonates who had pneumonia, all of the neonates who had bacterial growth in the oropharyngeal fluid aspirate also has bacterial growth in their blood. Those neonates who had no symptoms within the first 72 hours of life had no bacterial growth in their blood; however 4 of them have an isolate from the oropharynx. It is noteworthy that the bacterium isolated from their oropharynx is *Staphylococcus epidermidis,* which can be attributed as collection error probably because of aseptic technique.

## CONCLUSION

The sensitivity of oropharyngeal fluid aspirate culture irregardless of the organism isolated is 100%, the sensitivity is 72.35%. However this is not as reflective of the true sensitivity and specificity as there is a wide margin of error. Prelabor rupture of membrane has the highest risk of having bacterial growth in the neonates' oropharyngeal fluid. Patients with neonatal pneumonia who have bacterial growth in the oropharyngeal fluid have a high percentage of having bacteremia.

## RECOMMENDATION

A further study with a larger sample size to have a narrower margin of error is recommended.

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