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Pattern of maternal vaginal flora in labour and its effect on neonate

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Abstract
This prospective study was conducted at Krishna Institute of Medical Sciences, Karad from April 2012 to March 2012.The study included a total of 353 mothers in labour and 357 newborns (4 pairs of twins) born to them. A high vaginal swab was taken from all the mothers in the study to identify the pattern of vaginal colonization in labour. All their neonates were included in the study to determine the various neonatal outcomes like preterm birth or neonatal sepsis in regard to the vaginal colonization. All the neonates who developed sepsis were further admitted to the NICU, septic screening was done and were given appropriate treatment. Study conclude that knowledge of the pattern of maternal genital flora could help us to identify women at risk of preterm labour and neonates at risk of developing neonatal sepsis.

Keywords: labour, pregnancy, vaginal flora, neonate, intrauterine inflammation, childbirth

1. Introduction
Infection linked to pregnancy and childbirth has for centuries caused concern for women and their caregivers. Many strains of the neonatal microbiota arise during the first week of existence as a consequence of intrapartum access to maternal genital microorganisms [1]. The pattern of organism and the heaviness of colonization are usually closely similar between mother and the baby [2]. Intrauterine inflammation has been found to play a significant function in the development of preterm birth and neonatal sepsis [3]. Neonatal sepsis also remains one of India's major causes of neonatal mortality. The prevalence of neonatal sepsis is 30/1000 live births, according to the National Neonatal Perinatal Data (NNPD 2002-2003).

Aims and Objectives
To study the pattern of organism in the maternal vaginal flora of pregnant women in labour and to correlate its association with neonatal sepsis. To study the incidence of neonatal sepsis in Krishna Institute of Medical Sciences, Karad.

Review of Literature
P M Dunn (2004) in an article on 'prevention of puerperal fever' mentions that Ignac Semmelweis believed that washing with soap was not sufficient to remove all adhering particles. He insisted that child bed fever could be prevented by frequent hand washing with chlorinated lime water[4]. The current state of our understanding of the flora is that each woman is host to several anerobic and aerobic species simultaneously. The normal flora is also generally considered to prevent the attachment and growth of frankly pathogenic microorgansims and thereby protects the tissue from invasion [5].

Munson *et al*, (1982) reported in their study that 25 of 27 infants infected were premature and 16 were less than 29 weeks of gestational age [6].

A Mahmood *et al* (2002) carried out a study to determine the bacterial pathogens causing neonatal sepsis at a hospital at Karachi. Among 170 cases of early onset sepsis E coli was responsible for 19 (11.17%) cases. Out of 42 cases reported for late onset sepsis E coli caused 3 (7.14 %) cases [7].

Mai Jing Yun *et al* (2010) observed that the clinical manifestations of neonatal sepsis caused by klebsiella pneumonia are usually non-specific.CRP detection is valuable for early diagnosis of sepsis [8].

Manisha N *et al* (2012) in a study on emergence of multi-drug resistant klebsiella pneumoniae, reported their concern over the high prevalence of MDR species of klebsiella.Among the 110 infected neonates 23 cases were found to be due to klebsiella, 60.86% of them were fatal. Most of the isolates were to be more susceptible to amikacin, imipenem and piperacillin but were resistant to the third generation cephalosporins [9].

Subhashree Roy *et al* (2013) identified that feeding through an enteral tube was the only risk factor for sepsis by ESBL producing organisms [10].

Paul Nyirjesy and Jack D Sobel (2003) in their study on on vulvovaginal candidiasis have suggested the various factors that may be important in the development of vulvovaginal candidiasis. The various factors have been enlisted below [11].

Werner Mendling (2009) in his study on vaginal candida colonization and vaginal candidiasis during pregnancy has summarized that at least 30 % of all pregnant women are colonized with yeasts, of which 90 % are candida albicans. During vaginal delivery, vertical transmission of the yeasts occurs in 70 % to 85 % of cases.Thus the candida carriage rate among neonates varies between 22 % and 24 % depending on the frequency of cesarean sections. Even 90 % of the healthy and mature newborns develop clinical signs of oral thrush and /or anogenital candidiasis (diaper rash) within the first 12 hours. Most infants, however (10 %) show signs of infection

between the second and the fourth week post-partum [12].
Hypothermia is a common manifestation of sepsis in preterm babies, while term babies may manifest with fever, especially in gram positive infections. Diarrhea, vomiting and abdominal distention may precede or follow. Onset of jaundice after the age of 3 days and elevation of direct bilirubin to more than 2mg/dl is suggestive of associated hepatitis. Hepato splenomegaly may or may not be present. Episodes of apneic spells with cyanosis may be the sole manifestation of sepsis in preterm babies. In some babies, failure to gain weight or unexplained loss of weight may be the only manifestation [13].

Materials and Methods

This is a prospective analysis conducted at the Department of Obstetrics and Gynecology and the Neonatal Intensive Care Unit of Krishna Institute of Medical Sciences, Karad during the period of April 2012 to March 2013. The present study was carried out on 353 pregnant women in labour High vaginal swabs were taken from these women in labour at the first vaginal examination after taking informed consent. The vaginal swabs were taken from the posterior fornix with complete aseptic precautions using a speculum. A detailed history was obtained from the mothers which included the age, last menstrual period and expected date of delivery, ultra-sonographic findings in antenatal scans, obstetric history, presenting complains at labour, with premature rupture of membranes, associated disorders and drugs used during pregnancy. The neonates born to these mothers were further evaluated during their duration of stay in the hospital.
Balaka *et al* [14]. said that newborns are mainly infected by passage through a colonized birth canal at delivery. So the pattern of organisms in women with vaginal delivery were of more concern. There was no significant association between any of the organisms and the modes of delivery. No other study has evaluated the association of the pattern of organisms in the genital tract during labour in women with different modes of delivery.
In newborns born to mothers who were culture positive most of them were healthy and had no associated complications. Taking into consideration the neonatal morbidities in newborns of mothers with vaginal colonization preterm birth (7.97%) and sepsis (7.36%) were the commonest morbidities observed (Table 6). The Chi- square value was 21.325 and the P value was 0.0189. We found a significant association between babies born to culture positive mothers and prematurity and sepsis. Our findings are in corroboration with studies by other authors [2, 15, 16]. who have put forward a hypothesis that intrauterine infection has been shown to play a major role in induction of preterm birth.

Observations and Results

This is a prospective study conducted in the obstetric and Gynecology department and the Neonatal Intensive Care Unit (NICU) of Krishna Institute of Medical Sciences and Krishna Hospital, Karad from April 2012 to March 2013.There were 353 mothers in labour and 357 neonates (4 pairs of twins) included in the study during this period.

Table 1: Maternal Characteristics

Variables	N (n= 353)	Percentage (%)
Age		
18-25yrs	270	76.48 %
26-32yrs	72	20.39 %
≥33yrs	11	3.11 %
Parity		
Primigravida	95	26.9 %
Multigravida	258	73.08%
Gestational Age		
≤ 36 wks	18	5.09%
37-42 wks	283	80.1%
≥ 43 wks	52	14.7 %
Associated disorders		
PIH	37	10.4 %
Hypothyroid	1	0.28%
Hepatitis C	1	0.28%
Diabetes	3	0.84%
Heart disease	1	0.28%
Singleton /Doubleton pregnancy		
Single pregnancy	349	98.8%
Twin pregnancy	4	1.13 %

As seen in table no. 1, out of a total of 353 mothers included in the study, 270 (76.48%) were in the age group of 18-25 years. 258 (73.08%) mothers were multigravida. 283 (80%) were in the gestational age of 37-42(term) weeks. PIH was found in 37 (10.4%) mothers and was the most commonly associated disorder. 349 (98.8%) mothers had singleton pregnancy.

Table 2: Vaginal Swab Culture Results

Single / Poly organism	Culture positive (n= 160)	Culture negative (n= 193)
Culture with single organism	152 (95%)	0
Culture with poly organism	8 (5 %)	0
Total	160	0

As seen in table no. 2, out of a total of 353 mothers, 160 (45.3%) were culture positive and 193 (54.6%) were culture negative. Among the 160 culture positive mothers 152 (95%) mothers had single organisms and 8(5%) mothers had two organisms found in their culture respectively. Therefore a total of 168 organisms were found in mothers having positive culture results.

Table 3: Babies with Suspected Sepsis/Diagnosed Sepsis

Total number of babies in the study	Babies with suspected sepsis	Babies diagnosed with sepsis
357	32 (8.96 %)	14 (3.92 %)

As seen in table no. 3, out of the 357 babies included in the study,we had suspected sepsis in 32 babies. Only 14 (3.92%) of the 32 babies in whom the investigations/clinical signs were supportive of sepsis had been diagnosed as septic babies in the study further.

Table 4: Organism in Vaginal Culture and Sepsis in Newborn

Organism isolated from mothers vaginal culture	No of organisms isolated from mothers vaginal culture	Sepsis present in the newborn (n = 14)	Sepsis absent in the newborn	Fisher's exact P- value
Staphylococci	56	6 (42.8%)	50	0.0118
Streptococci	22	2 (14.2%)	20	0.2070
E coli	18	1 (7.1%)	17	0.5179
Klebsiella	15	1 (7.1%)	14	0.4541
Candida	51	2(14.2%)	49	1.0000
Citrobacter	2	0 (0%)	2	1.000
Diphtheroids	1	0(0%)	1	1.000
Pseudomonas	3	0(0%)	3	1.000
No organisms	193	2 (14.2%)	191	0.0044

As seen in table no. 4, the highest number (42.8%) of newborns with sepsis were born to mothers who had staphylococcus isolated in their vaginal swab culture. The highest number of organism isolated from the maternal vaginal culture was also staphy lococcus. The presence of staphylococcus in the maternal culture was significantly associated with the presence of sepsis in the newborn (The Fisher's exact P value was 0.0118).Similarly the absence of organisms in their vaginal culture was very significantly associated with the absence of sepsis in their newborns (The Fisher's exact P-value was 0.0044).

Table 5: Babies Who Died of Sepsis

Cause of death	Eos/ los	Organism found in baby's blood culture	Organism isolated from mother's vaginal culture	Birth weight of the baby
Severe sepsis with sclerema	Eos	E.coli	E coli	1.7kg
Sepsis with congenital heart disease	Los	Staphylococcus	Staphylococcus	1.7kg
Sepsis with meningitis	Eos	Sterile	Staphylococcus	2.8kg
Septic shock	Eos	E.coli	Staphylococcus	1.1kg
Septic shock	Eos	Staphylococcus	Staphylococcus	1.4kg

As seen in table no. 5, the causes of death of the 5 babies who expired due to sepsis were severe sepsis with sclerema, sepsis with congenital heart disease,sepsis with meningitis and sepsis with shock.4 and 1 out of the 5 babies had early onset and late onset sepsis respectively. 3 of the mother and baby-dyads had the same organism in their respective cultures. 4 out of the 5 babies had low birth weight.

Discussion

This study was undertaken to determine the pattern of vaginal tract colonization during labour, the maternal risk factors and their association with various neonatal complications. In our study 353 mothers were screened during labour for vaginal colonization. 160 (45.3%) of them tested culture positive. 152 (95 %) of them had single organisms while 8 were found to have two organisms at the same time. This is in support with the study by Carson *et al* who introduced the term compatibility profiling to describe the hypothesis of a dominant regulatory bacteria accompanied by co isolates [17].

In the present study there were 32 babies who were suspected of sepsis.There were 18 sick babies in whom their disease condition looked like sepsis but were proved otherwise with the support of investigations. Amongst such babies there were 13 babies with hypoglycemia, 2 babies with meconium aspiration, 2 with necrotising enterocolitis and 1 baby with intraventricular haemorrhage who were suspected but not diagnosed as sepsis as the investigations did not support the diagnosis. There were 14 babies who were diagnosed as sepsis by blood culture or clinically and have been reported in our study as babies with sepsis.

The most common organism isolated in mothers with vaginal colonization was staphylococcus which was also the same organism commonly isolated in babies with blood culture positive sepsis indicating vertical transmission as also observed by other authors. The presence of staphylococcus in the maternal culture was significantly associated with the presence of sepsis in the newborn (The Fisher's exact P value was 0.0118). Similarly the absence of organisms in their vaginal culture was very significantly associated with the absence of sepsis in their newborns (The Fisher's exact P-value was 0.0044). There was a positive association between the organism found in the mothers high vaginal swab and the organism isolated from the blood culture of their newborns who had sepsis. The importance of vertical transmission was suggested by Vidya Ayengar *et al* [18]. as they had documented 24 (1.3%) mother-baby dyads with vertical transmission of infection. Also important to be considered here is the fact that further typing of these bacteria could not be done due to limitations of our study. Typing of these bacteria would have been of great help in identifying the different strains of these bacteria and to understand which of the strains were more pathogenic. Unfortunately we could not determine if it was the same strain of these bacteria which was being transmitted from the mother to the baby.

Out of the 14 noenates with sepsis, 7(50%) of them were discharged owing to timely diagnosis and appropriate therapy. 5(35.7%) expired due to severe sepsis associated with meningitis, septic shock and associated co-morbidity such as

congenital heart disease. 2(14.2%) went DAMA due to financial constraints. We found an equal proportion of candida isolates as other etiologic pathogens of preterm birth such as staphylococcus and streptococcus. We also found a significant number (30%) of candida isolates in neonates who were delivered postdate. No other study has found such a correlation.

Conclusion

This study conclude that knowledge of the pattern of maternal genital flora could help us to identify women at risk of preterm labour and neonates at risk of developing neonatal sepsis. Staphylococcus, Streptococcus, E coli, Klebsiella and Candida were the most common organisms found in the vaginal culture of mothers in labour at our hospital Krishna Institute of Medical Sciences, Karad. Incidence of neonatal sepsis was 1.05 % during the study period at our hospital, Krishna Institute of Medical Sciences, Karad. Newborns of colonized mothers had higher chances of having infection than newborns of non-colonized mothers. Early onset-neonatal infection among newborns of mothers with vaginal colonization was significantly higher compared to newborns of mothers without vaginal colonization. Low birth weight and prematurity were significant risk factors for developing neonatal sepsis. Incidence of neonatal sepsis was higher in neonates delivered per vaginally indicating vertical transmission. Organisms causing neonatal sepsis in our neonatal intensive care unit (NICU) were Staphylococcus, Streptococci and E coli. The high vaginal swab of most of the mothers of babies with sepsis due to staphylococcus also showed the same organism in their vaginal culture results indicating vertical or perinatal transmission. CRP (C-reactive protein) is a reliable indicator of diagnosis of neonatal sepsis. According to the antibiogram profile of organisms causing neonatal sepsis in our NICU, most of the isolates were resistant to ampicillin. High sensitivity towards vancomycin and amikacin was shown by most of the gram positive isolates.E coli, a gram negative isolate showed high sensitivity only towards amikacin. Incidence of preterm birth was 4.3 % during the study period in our hospital, Krishna Institute of Medical Sciences, Karad. Risk factors like maternal vaginal colonization, pregnancy induced hypertension and twin pregnancy were significantly associated to premature birth.

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