PREVALENCE OF PATHOGENS IN VAGINA DURING PREGNANCY: RELATION BETWEEN THE MATERNAL GENITAL PATHOGENS AND EARLY ONSET NEONATAL BACTERIAL SEPSIS

Shrestha R[1], Nayak N[1], Shrestha J[2], Gokhale S[1]

Department of Microbiology, [2]Department of Obstetrics and Gynaecology Manipal College of Medical Sciences, Pokhara, Nepal

ABSTRACT

The maternal vaginal flora can be a source for neonatal sepsis. This study was undertaken to determine the prevalence of aerobic pathogens in vagina during third trimester of pregnancy and their role in causing neonatal sepsis. High vaginal specimens were collected from 150 participants attending Manipal Teaching Hospital from November 2014 to Aug 2015. Isolates were identified by standard microbiological methods and tested for in vitro antibiotics susceptibility by modified Kirby-Bauer disc diffusion method. Review of medical records was undertaken to determine the role of these pathogens in neonatal sepsis. The commonest pathogens recovered were Staphylococcus aureus 35 (38.8%), followed by E. coli 19 (21.11%), Candida albicans 14 (15.55%), Klebsiella pneumoniae 5 (5.55%) and Group B Streptococcus 4 (4.44%). High resistance to cefoxitin (20%) amongst Staphylococcus aureus and to ceftriaxone 15% and 20% in E. coli and Klebsiella respectively were recorded. Clinical sepsis was present in 10/24 newborns. Blood culture was undertaken in all 10 cases, which revealed pathogens in 6 cases. No bacteria were isolated from the blood culture of 4 neonates, despite clinical diagnosis of sepsis. The isolates, S. aureus (3), E. coli (2) and Klebsiella species (1), isolate from newborn were sensitive to Cefotaxime, Gentamicin and Amikacin. Four out of six isolates from the mother were phenotypically similar to corresponding neonates. Increased colonization of organisms is associated with high incidence of PV leaking which leads to with possibility to preterm delivery and emergency LSCS. Premature rupture of membranes and preterm labor are the leading risk factors for neonatal sepsis. Maternal vaginal flora has potential to cause neonatal sepsis. Presence of Candida albicans in vagina was associated with lower bacterial load as well as lower/no neonatal sepsis. Screening for the pathogens in vagina during late third trimester of pregnancy with history of leaking should is advocated.

KEYWORDS

Aerobic vaginal pathogens, high vaginal swab, neonatal sepsis, pregnancy

CORRESPONDING AUTHOR

Rajani Shrestha (Lecturer),
Department of Microbiology, Manipal College of Medical Sciences, Pokhara, Nepal
Email: _sth7@hotmail.com

INTRODUCTION

Vaginosis and vaginitis among pregnant women in third trimester can lead to numerous complications such as preterm labor, premature rupture of membrane, amnionitis and low birth weight of neonate associated with high perinatal mortality. Timely diagnosis and appropriate treatment can reduce the risk of the aforementioned consequences.1 This study was conducted to determine the prevalence of aerobic pathogens in vagina during third trimester pregnancy, their transmission to newborn during delivery, and the possible impact of such colonization on the occurrence of neonatal sepsis.

MATERIALS AND METHODS

Study design:

This study was carried out at Manipal Teaching Hospital (MTH), Pokhara from November 2014 to Aug 2015. Participants were in third trimester of pregnancy.

Inclusion criteria: Pregnant women without any history of antimicrobial therapy within preceding 15 days, attending antenatal care or delivery at MTH.

Exclusion criteria: Pregnant women with high risks like placenta previa, history of eclampsia and/or with systemic illness, antimicrobial therapy within preceding 15 days.

Demographic information of the participants was obtained by questionnaire. Relevant information regarding the age, parity, socio-economic status, antenatal visit, and personal hygiene practices was recorded in the proforma. Personal hygiene status was graded in three groups according to criteria and points:2 Group 1: vigorous personal hygiene practices, Group 2 moderately vigorous personal hygiene practices and Group 3 non-vigorous personal hygiene practices.

Specimen collection and processing:

A total of 150 high vaginal specimens were collected by sterile cotton swabs after obtaining informed consent from both in-door and out-door participants. The samples were processed immediately in the microbiology laboratory. The specimens were subjected to direct microscopy by Gram's stain and culture. Mac Conkey's agar was incubated aerobically overnight at 37ºC. Blood agar and chocolate agar plates were incubated at 37°C in presence of 5-10% CO_2. Plates were examined for any growth after 24 and 48 hours of incubation. Bacteria were identified based upon their colony morphology, staining and biochemical properties using the standard protocol.3 Antibiotic susceptibility pattern was determined by Kirby-Bauer disc diffusion method using Muller Hinton agar and sheep blood agar following the criteria designed by the Clinical and Laboratory Standards Institute. (2013) Septic screening (complete blood counts (TLC, DLC, Platelet count, C-reactive protein) and bacteriological culture of blood was undertaken in all participants and neonates clinically suspected to have sepsis. The medical records of all participant mothers and neonates were analysed to collect relevant information.

RESULTS

Amongst the participants, 116 (77.3%) were primigravida and 34 (22.7%) were multigravida. Youngest was 18 years and oldest was 34 years age. Large numbers, (80%) of participants were below 29 years age. Twenty-four (16%) participants were uneducated, 84 (56%) had completed SLC, 17 (11.3%) had passed higher secondary, 24(16%) were graduate, and only 1 (0.6%) was postgraduate. Among the participants 66% (n=99) had regular ANC visit whereas 34% (n=51) had irregular ANC visits. In this study, only 25% of the participant who were uneducated complied with regular ANC visits whereas 75% had irregular visits. Educated participants fully complied with ANC visits schedule.

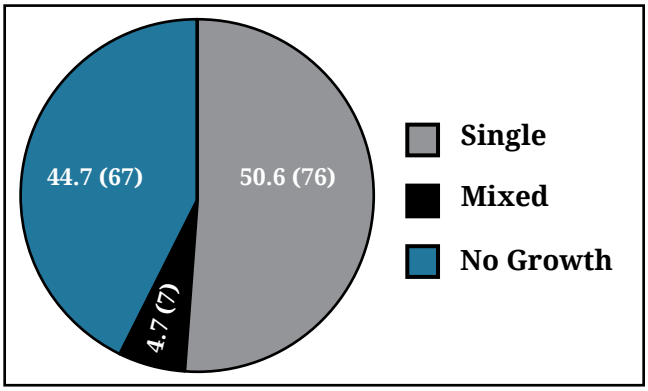

Fig. 1: Distribution of microorganisms isolated in single and mixed culture from vaginal swab specimen.

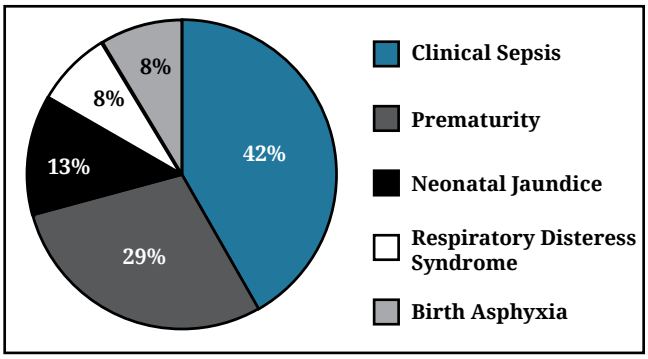

Fig. 2: List of causes of admission in NICU.

Participant who had non-vigorous personal hygiene practices were (19/150; 12.66%), moderately vigorous personal hygiene practices (129/150; 86%) and vigorous personal hygiene practices (2/150; 1.33%). Out of 150 high vaginal swabs processed, 83 (55.33%) showed bacterial or yeast growth on culture. Of these 83 culture positive samples, 76 (91.5%) yielded single organisms whereas 7 (8.4%) resulted in two types of organism,

amounting to 90 isolates. (Fig. 1)

Staphylococcus aureus (35/90; 38.8%) was the most predominant organism followed by *E coli* (19/90; 21.11%), and *Candida albicans* (14/90; 15.55%). Other organisms such as Coagulase negative Staphylococcus (CoNS) (6/80; 6.6%), *Klebsiella pneumoniae* (5/90; 5.55%), Group B *Streptococcus* (4/90; 4.44%), *Enterococcus* (4/90; 4.44%), *Proteus mirabilis* (3/90; 3.33%) were isolated in smaller numbers. (Table No. 1)

Table 1:

Organism isolated	Number of isolates	Percentage (%)
Gram Positive Organisms:		
Staph aureus	35	38.8%
a) MRSA	18	20%
b) MSSA	17	18.8%
CONS	6	6.6%
Enterococcus faecalis	4	4.44%
Group B Streptococcus	4	4.44%
Gram Negative organisms:		
Escherichia coli	19	21.11%
Klebsiella pneumoniae	5	5.55%
Proteus mirabilis	3	3.33%
Fungi:		
Candida albicans	14	15.55%
Total	**90**	**100%**

Paranthesis represent percentage.

Fifty one percent (18/35; 51.4%) *S aureus* were methicillin resistant, while 48.5% (17/35) were methicillin sensitive. The isolation rates of Candida were significantly higher among women with non-vigorous personal hygiene practices, (6/19; 31.57%) as compared to those with moderately vigorous personal hygiene practices (3/129; 2.32%) and vigorous personal hygiene practices (0/2; 0%).

The mode of delivery was vaginal in 86% (129/150) while 8% (12/150) underwent emergency LSCS and 6.0% (9/150) underwent elective LSCS. In this study, 16.7% (25/150) had history of PV leaking and 83% (125/150) did not have leaking. Seventy two percent (18/25) participants with history of PV leaking revealed bacterial or candida colonization as compared to only 52% (65/125) participants with no history of PV leaking revealed the colonization ($p< 0.05$). This difference was found to be statistically significant suggesting that the higher rate of colonization was associated with incidence of PV leaking.

Twenty four percent (6/25) participants with history of PV leaking but only 4.8% (6/125) with no history of PV leaking underwent emergency LSCS. Similarly, 28 % with the history of PV leaking had preterm delivery, whereas 10.4% with no history of PV leaking had preterm delivery. The occurrence of preterm delivery was higher in those with PV leaking (Table 2).

Of the total of 150 deliveries, 24 neonates were admitted to ICUs. Besides clinical sepsis (10), the newborns were diagnosed with Prematurity (7), Neonatal jaundice (3), Respiratory distress syndrome (2) and Birth asphyxia (2) (Figure No 2). Blood cultures were undertaken in all 10 cases as a part of septic screening. Pathogens were isolated from 6/10 blood samples showed growth (Table No 3). The isolates from high vaginal swabs of four mothers and corresponding neonates were similar by biotyping and antibiogram, indicating transfer of organism from mother to neonate during delivery (Table 3 S.No.1, 2, 3 & 5).

Table No. 3

S. No.	Participants No.	Isolation from mother	Isolation from neonate	Mode of delivery
1.	116	MRSA	MRSA	Preterm delivery (History of leaking)
2.	118	MSSA	MSSA	Vaginal (term) delivery (History of leaking)
3.	126	MRSA	MRSA	Emergency LSCS (term) (History of leaking)
4.	103	*E. coli*	*K pneumoniae*	Preterm delivery (History of Leaking)
5	78	*E. coli*	*E. coli*	Preterm delivery (History of leaking)
6	106	No growth	*E. coli*	Preterm delivery (History of Leaking)
	Total	5	6	

Table No. 2

	Organisms isolated					
	No Growth	Gram Positive bacteria	Gram Negative bacteria	Fungi	Poly Microbial	Total (%)
PV Leaking absent (n=125)						
Emergency LSCS	1	2	2	1	0	6 (4.8)
Elective LSCS	4	3	0	0	1	8 (6.4)
Normal delivery	50	28	12	5	3	98 (78.4)
Preterm delivery	5	5	1	0	2	13 (10.4)
Total	60	38	15	6	6*	
PV leaking present (n=25)						
Emergency LSCS	1	1	3	1	0	6 (24)
Elective LSCS	0	0	0	1	0	1 (4)
Normal delivery	4	3	3	1	0	11 (44)
Preterm delivery	2	3	1	0	1	7 (28)
Total	7	7	7	3	1*	
Grand total (with and without PV leaking)						
Emergency LSCS	2	3	5	2	0	12 (8)
Elective LSCS	4	3	0	1	1	9 (6)
Normal delivery	54	31	15	6	3	109 (72.66)
Preterm Delivery	7	8	2	0	3	20 (13.33)
Total no of participants	**67**	**45**	**22**	**9**	**7**	**150**

Parenthesis represents percentage; * two type of organisms isolated.

DISCUSSION

The primary goal of this study was to identify the aerobic pathogens that might be present in the vaginal tract and their impact, if any, on the outcome of pregnancy. Review of world literature revealed few studies that were conducted to determine the presence of bacteria in the vaginal tract.[4,5,6] However, data from Nepal is scanty.[7]

In this study, young sexually active females of 20-24 year age group predominated (74/150; 49.3%) These findings suggest that early marriage and early pregnancy are common in Nepal. However, more organisms were isolated in above 29 years group (11/16) followed by 20-24 years (43/74), < 20 years age (8/15), and then 25-29 years (21/45). These findings indicate that the isolation of organisms doesn't depend on the age groups but are related to the ecosystem of vagina during pregnancy.

In the present study, *S aureus* was the most prevalent organism followed by *E coli*, *Candida albicans*, Coagulase negative Staphylococcus, *Klebsiella pneumoniae*, Group B *Streptococcus*, *Enterococcus* and *Proteus mirabilis* in the study. Among the isolates of *S aureus* 20% were methicillin resistant and 18.8% were methicillin sensitive. All MRSA were sensitive to vancomycin (Va) and amikacin (Ak). Both MRSA and MSSA isolates exhibited variable resistance to penicillin, gentamicin, ciprofloxacin and ceftriaxone (94.4%, 27.8%, 33.3%, 44.4% MRSA) and (88.2%, 11.8%, 17.6%, 23.5% MSSA). Such high rate of penicillin resistance amongst both MRSA and MSSA strains could be because of the indiscriminate use of antibiotics.[8]

The second most prevalent organism isolated from the high vaginal swabs was *E coli* (21.11%). Anatomical proximity of urethra and vagina to anus predisposes spread of colonic *E coli* to vagina and urethra leading to recurrent infections. Poor hygiene practices and the inability to cleanse the genitourinary areas may also precipitate spread of organisms.[9] All *E coli* were sensitive to Imipenem and Amikacin. In this study, 15% *E coli* and 20% *Klebsiella* were resistant to ceftriaxone (third generation cephalosporin). These could be Extended-spectrum β-lactamases (ESBL) producers. During the past decade, ESBL producing *E coli* and *K pneumoniae* have emerged as serious pathogens.

Rising incidence of infections due to ESBL producing strains has become a worldwide problem resulting in limited therapeutic options. Vaginal carriage of *E coli* could represent a real threat of maternal genital as well as neonatal infections. Maternal lower genital infection might mimic bacterial vaginosis, albeit their unresponsiveness to antibacterial vaginosis medication.[10]

Group B *Streptococcus* (GBS) 4/90 (4.44%) was isolated in this study. All the strains of *Streptococcus* were sensitive to penicillin and vancomycin. GBS colonization rates in pregnant women have been reported to range from 2% to 30%.[11] Maternal group B *Streptococci* (GBS) colonization is a major risk factor for amnionitis, endometritis and stillbirth; occasionally leading to meningitis in neonates.[3]

Prevalence of *Candida* species was 15.55 %. Candida was detected in 6/19 (31.57 %) of women with non-vigorous personal hygiene practices as compared to only 2.32% of women with moderately vigorous personal hygiene practices, but none from participants having vigorous personal hygiene practices. This difference was found to be statistically significant. This is akin to the established notion that prevalence of *C albicans* is dependent on the physiological changes such as high estrogen and glycogen content of the vaginal mucosa providing adequate supply of utilizable sugar that favor candidal growth during pregnancy.[12] Comparatively higher rates of detection of *C albicans* could be related to low socio-economic and educational status, and noncompliance for scheduled visit for antenatal checkup. This is reflected in our observation that as high as 18/24 (75%) of the women who were in the low education category had poor compliance to antenatal advice. When candida was isolated from mother, only 11.1% neonates were cared at NICU and 88.8% were cared at home. This reflects that candida colonization in vagina leads to less chances of neonatal sepsis compared to other gram-positive bacteria (27.2% neonates admitted in NICU, while 72.7% neonates were cared at home) and gram-negative bacteria (20% neonates admitted in NICU, 80% neonates were cared at home).

In the present study, pathogens from high vaginal swab were detected among 72% of women with PV leaking in contrast to only 52% women without any history of PV leaking (Table No 1) suggesting thereby that higher rate of vaginal colonization was associated with PV leaking. Majority (86%) of pregnant women underwent vaginal delivery while 8% had emergency LSCS and 6% required elective LSCS. In this study, the indications for emergency LSCS were fetal distress, severe oligohydraminos and non-progressing labor. Indications for elective LSCS were previous Caesarean Section (CS), breech presentation, CS on demand and CPD (cephalo-pelvic disproportion).

In this study, *E coli* 5/25(20%) and *S aureus* 7/25 (28%) were isolated from the vagina of pregnant woman with PV leaking. This could have been due to the proximity of vagina to perianal region and improper cleansing of the genitourinary areas.13 Premature Rupture of Membrane (PROM) may lead to preterm delivery, neonatal infection (pneumonia, meningitis, sepsis), umbilical cord compression, abortion and cord prolapse.14 Previous studies showed good correlation between genital tract flora and organism grown in amniotic fluid or blood of neonates with early onset sepsis. Bacteria can be transmitted to the neonate from mother's blood, skin, and vaginal tract before or during delivery. Several studies reported *Streptococcus agalactiae*, as the most common causative organism for neonatal sepsis, followed *E coli, Klebsiella* spp. and *S aureus*.15,16

In our study, premature rupture of membranes and preterm labor were the leading risk factors for neonatal sepsis. Neonatal ICU admissions were due to clinical sepsis (10), prematurity (7), neonatal jaundice (3), respiratory distress syndrome (2) and birth asphyxia (2). Clinical sepsis was present in 10 newborns (41%) of the total neonates admitted. In all these 10 cases, blood samples were taken for culture as a part of sepsis screening which revealed positivity in 6 cases (3 *S aureus,* 2 *E coli* and 1 *Klebsiella* species). These six organisms were sensitive to cefotaxime, gentamicin and amikacin.

We compared the profile of the organisms isolated from the blood of neonates with those of the isolates from the HVS of corresponding mothers. In 4 out of 6, the organisms isolated from the mother and neonates were the same with similar biotyping and antibiogram (Table No. 3) while the remaining four blood cultures showed no growth. Conventional blood cultures are considered being the 'gold standard' for the detection of microbial pathogens in sepsis cases. Lower sensitivity of culture methods could be the reason for non-isolation of bacteria from 4 cases, despite strong clinical suspicion of neonatal sepsis.

Conclusion: This study showed significant prevalence of aerobic pathogens in vagina during pregnancy. Participants with non-vigorous personal hygiene practices more often have polymicrobial colonization. Maternal vaginal flora has potential to cause neonatal sepsis. The main pathogens harboured were *S aureus*, *E coli* and Group B *Streptococcus*. Candida colonization in vagina, compared to bacterial colonization, leads to lesser chances of neonatal sepsis. The candida perhaps offers protection against colonization by bacterial pathogens. The incidence of PV leaking was associated with the increased isolation of organisms with possibility of preterm delivery and emergency LSCS. Premature rupture of membranes and preterm labor are the leading risk factors for neonatal sepsis. Screening for the pathogens in vagina during late third trimester of pregnancy with the history of PV leaking should is advocated.

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Author's contribution

Rajani Shrestha, designed the study, collected and processed the specimen, analysed data and drafted manuscript while Dr. JSS Sharma and Dr. Junu Shrestha refined the study protocol and specimen collection methods. Dr Shishir Gokhale, Dr Niranjan Nayak and Dr. JSS Sharma distilled the manuscript. All authors have read and accepted the manuscript.

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