

## PREVALENCE OF PERINATAL INFECTION IN SOUTHERN ODISHA, INDIA.

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**ABSTRACT: AIM:** This study was undertaken to evaluate the prevalence of perinatal bacterial infection in southern part of Odisha. **MATERIALS AND METHODS:** Pregnant women presenting with premature rupture of membrane (PROM), preterm labour, fever, vaginal discharge (VD), urinary tract infections and previous bad obstetric history were included in the study groups. Besides newborns < 7 days old admitted to Paediatrics Department were also included. High vaginal swab and blood sample were collected from mother whereas from neonate cord blood and umbilical swab were taken. **METHODS:** Microscopy, culture for aerobic bacteria and serology for *C. trachomatis* were done. **RESULT:** Total number of cases proven to have microbiological infections based upon laboratory results. Maximum percentage of PNI cases were primigravida belonging to the age group of 20-30years and most common bacterial infections were caused by *S. aureus* & *C. trachomatis*. Ampicillin, Cefuroxime & Gentamicin were found to be the most effective antibiotics for bacterial isolates.

**KEYWORDS:** PROM – premature rupture of membrane, VD – vaginal discharge, PNI – Perinatal infection

**INTRODUCTON: Perinatal infection (PNI)** means the infection transferred from the mother to the baby after 28<sup>th</sup> week of gestation and up to 7 days after delivery. <sup>[1, 2]</sup> These infection leads to suffering, incapacitation, occasional permanent disability of child and also maternal and neonatal morbidity and mortality. Still it is one of the most neglected aspects of women and child health care. The global incidence of neonatal sepsis is 10-20 per 1000 live births. <sup>[3]</sup> In India the incidences of sepsis is 2-10 cases per 1000 live births and have a high risk of mortality approximately 25-30% i.e. about up to 7, 50,000 deaths every year. <sup>[3]</sup> Data from national neonatal and perinatal data base 2000 suggests that *Escherichia coli*, *Klebsiella* spp. and *Staphylococcus aureus* are the common causes of neonatal sepsis in India. The perinatal infection affects neonates widely in various ways. Infection is the most important cause of premature rupture of membrane, preterm labour and pPROM all of which lead to low birth weight baby. Due to low immune status, a newborn is highly vulnerable to infections. The PNMR

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(Perinatal mortality rate) reflects the quality of medical care. The PNMR in developing countries is three fold to five fold higher than in the developed countries.

**MATERIALS & METHODS:** The prospective study was carried out, in the Department of Microbiology, MKCG Medical College Hospital, Southern Odisha, India over a period of 22 months, in collaboration with departments of Obstetrics/Gynaecology & Paediatrics.

**CASE SELECTION:** 150 pregnant women admitted to the labour room, belonging to the age group of 15 to 40 years. The study group included females with premature rupture of membrane, preterm labour, fever, vaginal discharge, UTI and previous bad obstetric history. A matching group of 100 patients without such complaints admitted to labour room for safe confinement were included as controls. The newborns (<7 days) born to the pregnant women & subsequently admitted to Paediatrics Dept. and NICU were also included in the study.

**SPECIMEN COLLECTION AND PROCESSING: MOTHER:** Three high vaginal swabs/endocervical swabs were collected from the patients at the time of admission to labour room. One swab was used for direct microscopic examination, another swab was put in 0.9% saline for direct wet mount microscopy and yet another swab was used for aerobic isolation of micro-organism. A blood sample of 3 ml was collected from patients for serological study of *C. trachomatis*, and 5 ml blood for culture in puerperal sepsis.

**NEONATE:** Two umbilical swabs were collected for direct microscopy and aerobic isolation of organisms by culture after 24 hrs of birth. 2ml of blood collected for culture in neonatal septicaemia.

**ANTIBIOTIC SUSCEPTIBILITY TESTING:** Antibiotic susceptibility testing was performed by Kirby and Bauers standard disc diffusion method.

**SEROLOGY:** Out of 150 cases 60 cases with bad obstetric history were subjected for IgM ELISA test (By Novatec *C. trachomatis* IgM ELISA test kit) for *C. trachomatis*.

**RESULT:** Maximum cases were primigravida belonging to the age group 26 – 30 years followed by 21-25 years. Least number of cases was primigravida belonging to the age group 36-40 years. Maximum numbers of neonates were born from primigravida belonging to the age group 26-30 years (TABLE-I).

PROM, 43 (28.6%) was the single most common presentation in these patients (Fig: 1). Out of 150 cases, gram's staining revealed gram positive cocci in 50 (33.3%), gram negative bacilli in 35 (23.3%), clue cells in 28 (18.6%), and gram positive budding yeast cell in 10 (6.6%). Among 100 controls, gram positive cocci, gram negative bacilli, clue cells and gram positive budding yeast cell were found to be 18%,5%, 5% & 2% cases respectively (Fig: 2).

*S. aureus* (28%) was found to be the most common pathogen on culture isolation, followed by *E. coli* (20%) in the cases. In control group *S. epidermidis* (25%) was the most commonly isolated bacteria (TABLE-II).

Gentamicin is the most sensitive drug for gram positive isolates in new born where as ampicillin was the most effective drug followed by azithromycin & penicillin in mother (TABLE-III).

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All gram-negative bacilli showed 100% resistance to ampicillin in mother. Cefuroxime was the most sensitive drug followed by cephalexin and cefepime. Gentamicin is the most sensitive drug for gram negative isolates in new born (TABLE-IV).

16 (26.6%) out of 60 cases were positive for *C. trachomatis* by Ig M ELISA test and only 1 (5%) out of 20 controls were showing seropositivity (Fig.3).

**DISCUSSION:** Microbial flora of the female genital tract presents as an extreme and diversified spectrum of pathogenic and non-pathogenic organisms. While *Chlamydia trachomatis* and Group B  $\beta$ - hemolytic *Streptococcus* are associated with PNI, bacterial vaginosis caused by various other pathogens, has emerged as another risk factor for PNI.<sup>[4]</sup> In this study association of selected bacterial pathogens present in the mother and transferred to baby in the causation of PNI has been studied. In our study, the proportion of antenatal mothers attending labour room were high in the primigravida belong to the age of group 26-30 years (Table-1). This is comparable with the study of I. Jehan et al. where participants were predominantly in the age group of 19-24 years. The age distribution of control was the same as that in antenatal mother with PNI.<sup>[5]</sup>

Lower age incurs an increased risk of PNI because of biological and behavioral risk factors. Adolescents tend to have cervical ectopy, which provides large zones of columnar epithelium for the targeted attachment of *C. trachomatis* and *N. gonorrhoea* <sup>[4]</sup>. The present study showed the comparable observation (Table-1).

As in our study maximum no. of cases and controls were primigravida, the numbers of neonates were born maximum from primigravida belong to age group 26-30 years (Table-1). Premature rupture of membrane was the most common symptom in cases (28.6%) found in our study (Fig-1). The other symptoms and signs were preterm labour, pPROM, vaginal discharge, puerperal sepsis etc.

In our study, direct microscopic observation by Gram's stain revealed gram positive cocci in cases 50 (33.3%), gram negative bacilli 35 (23.3%) & gram negative cocci 4 (2.6%) (Fig-2). As per Amsel's criteria for the diagnosis of *G. vaginalis*, clue cell were found in 28 (18.6%) cases out of 150 (Fig-2).<sup>[5, 6]</sup> They found that sexually transmitted cofactor may strengthen, the relation between bacterial vaginosis associated microorganisms and development of PNI. In some other studies the prevalence of bacterial vaginosis was reported to be 6-32%.<sup>[7]</sup> In our study bacterial vaginosis associated bacteria *G. vaginalis* was identified as gram variable pleomorphic coccobacilli mostly in clumps. Deborah Money <sup>[8]</sup> also used gram stain of vaginal fluid for the diagnosis of bacterial vaginosis in lieu of clinical criteria, given its greater reliability for diagnosis of bacterial vaginosis. A number of studies proved that apart from *C. trachomatis*, bacterial vaginosis has been a risk factor for preterm labour, premature rupture of membrane, preterm premature of membrane.<sup>[5, 6]</sup>

Gram - negative diplococci were found to be 4 (2.6%). They were diagnosed to be *N. gonorrhoeae* morphologically by gram stain, as the culture was very cumbersome and not done. Bacterial culture findings of this study revealed isolation of *S. aureus* (28%) followed by *E. coli* (20%), Group B  $\beta$ - hemolytic streptococci (2.6%) and *Enterococcus* spp. (2.6%), *Klebsiella* sp. (1.3%) and *Pseudomonas aeruginosa* (1.3%). Least was *Proteus mirabilis* (0.6%), but in control group maximum isolates were *S. epidermidis* (25%) (Table-2). As compare in neonates the isolates were of *S. aureus* (17%), Group B  $\beta$ - hemolytic streptococci (1.3%), *E. coli* (8.8%), *Klebsiella* spp. (1.3%) and *Pseudomonas aeruginosa* (0.6%) (Table-2).

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Serological tests were generally not useful in the diagnosis of genital tract infection caused by *C. trachomatis* and that the presence of immunoglobulin M (Ig M) antibodies was an unreliable marker of acute infection in adolescents and adults. Numazaki K et al reported that the rates of Ig M seropositivity for *C. trachomatis* during pregnancy were significantly higher in mothers who had given birth to infants with complications than in matched controls.<sup>[9]</sup> The frequency of chorioamnionitis and meconium stained amniotic fluid was also higher in the anti *C. trachomatis* Ig M antibody-positive pregnant women. Several investigations have reported that 2-20% of pregnant women harbour *C. trachomatis* in the endocervix. Ig M antibodies on the other hand may be short-lived. ELISA tests of patients suspected with *C. trachomatis* infection have recently been used as a diagnostic tool. Hence, we used this test to demonstrate Ig M antibodies in cases of clinically diagnosed PNI. Out of 60 cases, 16 cases (26.6%) were found to be seropositive for *C. trachomatis* (Fig-3). Only 1 (5%) seropositivity was found in control group (Fig-3).

Our data confirms the relationship between maternal infection and perinatal outcome and support the hypothesis that infections might be the cause of these conditions and not simply an association. For preventing perinatal infections, antenatal mothers should be screened and treated for these infections. From our study most of the gram positive cocci isolated were found sensitive to ampicillin, penicillin and azithromycin (Table-III). All the gram negative bacilli show 100% resistance to ampicillin. Most sensitive drug for gram negative bacilli is cefuroxime (Table- IV). Gentamicin was the most effective antibiotic for both gram positive cocci and gram negative bacilli in neonates (Table-III & IV).

**CONCLUSION:** The study was carried out over a period of 22 months in the Dept. Of Microbiology, M.K.C.G. Medical College, Southern Odisha, India. All the samples collected from both the cases and controls were subjected for bacteriological & serological analysis. In view of the above finding available from results in our study we could derive the following conclusions: incidence of PNI attending labour room was highest in primigravida belonging to the age group 26 – 30 years. *Staphylococcus aureus* and *C. trachomatis* were the most common aetiological agent of PNI. Other bacterial pathogens isolated in the culture were Gr. B  $\beta$  hemolytic Streptococci, Enterococci, *E. coli* and *Klebsiella* spp. Ampicillin, azithromycin, cefuroxime and gentamicin were found to be effective on the bacterial isolates.

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**TABLE-I: Age distribution of antenatal mother and neonate of study group**

Age (years)	Cases			
	Mother		Neonate	
	Primigravida	Multigravida	Primigravida	Multigravida
15-20	14	6	14	7
21-25	20	10	18	10
26-30	28	16	27	16
31-35	16	22	16	23
36-40	4	12	4	12

**TABLE-II: Culture isolation of different bacteria in antenatal mother and neonate.**

Bacteria	Mother		Neonate
	Cases	Control	
<b>GPC</b>			
S. aureus	42(28%)	15(15%)	25(17%)
S. epidermidis	12(8%)	25(25%)	4(2.6%)
GBS	4(2.6%)	0	2(1.3%)
Enterococci spp.	4(2.6%)	0	0
<b>GNB</b>			
E. coli	29(20%)	5(5%)	13(8.8%)
Klebsiella spp.	2(1.3%)	0	2(1.3%)
Pseudomonas aeruginosa	2(1.3%)	1(1%)	1(0.6%)
Proteus spp.	1(0.6%)	0	0

**TABLE-III: Sensitivity pattern of GPC to various antimicrobials.**

BACTERIA	MOTHR									NEONATE								
	Total no	P	A	Ox	Ch	Cp	Cu	At	Cd	Total no	A	Ak	G	Cf	Co	E	Cu	Ci
S. aureus	42	14	25	12	2	16	13	26	13	25	5	3	15	7	8	2	11	10
GBS	4	3	4	3	0	1	0	0	0	2	2	0	0	0	2	2	1	0

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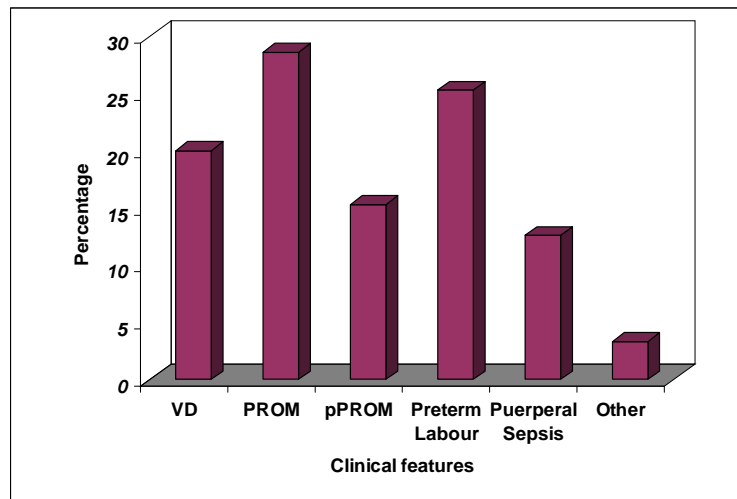
Enterococcus	4	2	1	0	2	0	2	3	2	1	0	0	0	0	1	0	1	1
Total no	50	19	30	15	4	17	15	29	15	28	7	3	15	7	11	4	13	11

(P – Penicillin, A – Ampicillin, Ox – Oxacillin, Ch – Cephalothin, Cp – Cephalexin, Cu- Cefuroxime, At – Azithromycin, Cd – Clindamycin, Ak – Amikacin, G – Gentamicin, Cf – Ciprofloxacin, Co – Cotrimoxazole, E – Erythromycin, Ci – Ceftriaxone)

**TABLE-IV: Sensitivity pattern of GNB to various antimicrobials**

BACTERIA	MOTHER						NEONATE								
	Total no	A	Cp	Cu	Cpm	At	Total no	A	Ak	G	Cf	Co	Ce	Cu	Ci
E. coli	29	0	22	13	11	11	13	2	8	9	3	4	2	5	1
Klebsiella	2	0	1	2	1	0	2	0	1	2	1	2	1	0	0
P.aeruginosa	2	0	0	2	1	1	1	0	0	1	0	0	1	1	0
P.mirabilis	1	0	1	1	0	0	0	2	0	0	0	0	0	0	0
Total no.	34	0	24	18	13	12	16	2	9	12	4	6	4	6	1

(A – Ampicillin, Cp – Cephalexin, Cu – cefuroxime, Cpm – Cefepime, At – Azithromycin, Ak – Amikacin, G – Gentamicin, Cf – Ciprofloxacin, Co – Cotrimoxazole, Ce - Ceftazidime, Ci – Ceftriaxone)



**Fig-1: clinical features of the patients**

(\*VD: Vaginal discharge, \*\*PROM: Premature rupture of membrane, \*\*\*pPROM: Preterm premature rupture of membrane.)

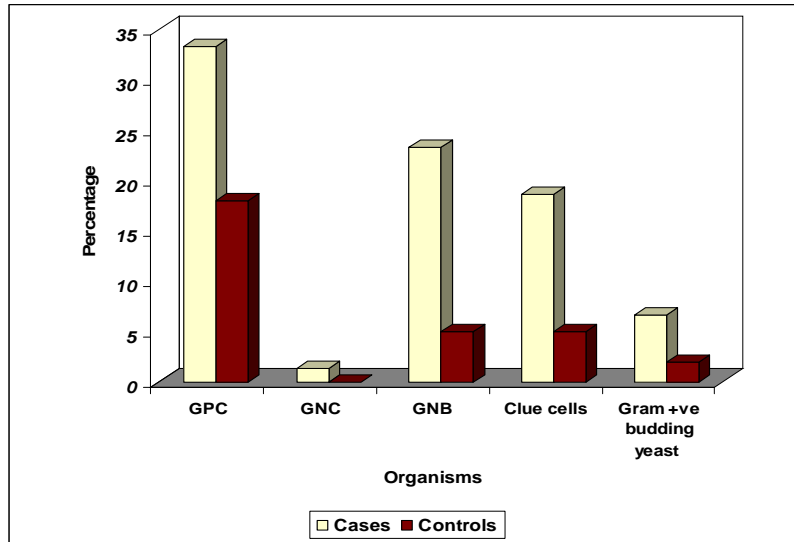


Fig-2: Microscopic observation by gram's stain method.

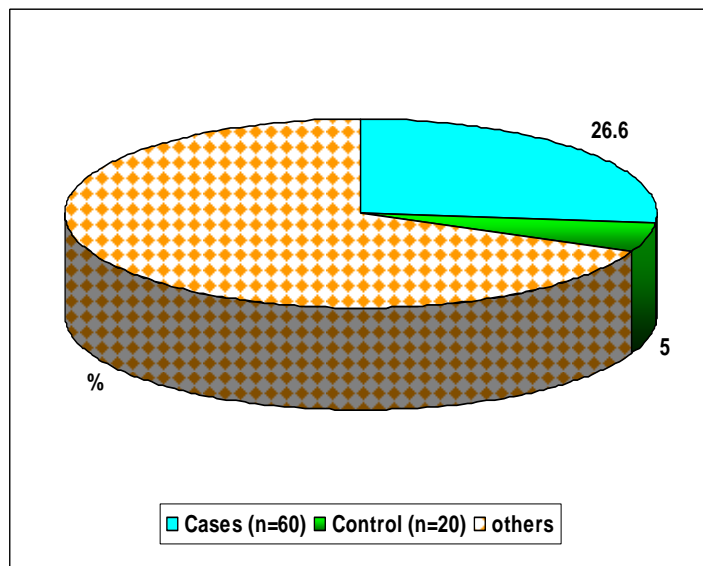


Fig. 3: Ig M ELISA test for Chlamydia trachomatis