

## Original Research Article

# Incidence of colonization of preterm neonates' gastric aspirate and its correlation with neonatal, maternal and environmental risk factors

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### ABSTRACT

**Background:** Infants born with Very Low Birth Weight (< 1500 grams) and Extremely Low Birth Weight (< 1000 grams) are at a high risk of pre-discharge morbidities. Aim of our study was to know the incidence of colonization of the preterm neonates' gastric aspirate and to co-relate it with the various neonatal, maternal and environmental risk factors.

**Material and Methods:** This was a prospective, observational study in 100 neonates conducted over a period of two years at Pediatrics Department of a teaching hospital of Western Gujarat region. All pre-term newborns who did not have any clinical features for sepsis were included in the study.

**Results:** In our study, most common organisms isolated were enterococcus (41%) followed by bacteroids (26%) and staphylococcus (14%) in 24 hours & 72 hours of gastric aspirate sample. Lower gestation age and low birth weight were significantly associated with colonization of the preterm neonatal gut ( $p < 0.05$ ). The positive colonization was also significantly correlated to low APGAR score. Highest incidence of neonatal sepsis was seen with enterococci organism (14.6%), where six newborns with enterococci isolates in gastric aspirate had developed neonatal sepsis, followed by *E. coli* isolates (12.5%).

**Conclusions:** We found a high incidence of colonization of the preterm neonates' gut, with enterococcus being the most common isolate. A significant association was observed between gut colonization and development of neonatal sepsis. Significant factors for development of gut colonization were: lower gestation age, multiple per-vaginal examinations and low birth weight of the newborns.

**Key Words:** Enterococcus, Gastric aspirate, Intestinal bacteria, Neonates, Neonatal Sepsis

### INTRODUCTION

Globally, prematurity is one of the leading causes of under-five deaths. More than 1 in 10 of the world's infants are born too early every year. Almost 1 million children die each year due to complications of preterm birth (PTB).<sup>1</sup> PTB is the leading cause of neonatal deaths and India is the biggest contributor to the global burden of PTB. Unfortunately, despite all the efforts, India has the highest number of deaths due to PTB accounting for 35% of neonatal deaths and ranks 36th globally.<sup>2</sup> A number of risk factors for PTB have been identified; including a prior history of PTB, underweight mothers, obesity, diabetes,

hypertension, smoking, infection, maternal age, genetics, multiple pregnancy, and pregnancies spaced too closely.<sup>2</sup>

Preterm babies are born before 36 weeks 6 days of gestational age. The care of preterm neonates is both labour intensive and consumes a major portion of neonatal intensive care unit (NICU) resources.<sup>3</sup> The very preterm neonates born less than 32 weeks gestation comprise 15.6% of preterm births and have a high risk of mortality and morbidity.<sup>4</sup> The survival till discharge is reported to be less than 80% for the very preterm infants and very low birth weight (VLBW) infants.<sup>5</sup>

The survivors of infants born with Very Low Birth Weight (VLBW) (< 1500 grams) and Extremely Low Birth Weight (ELBW) (< 1000 grams) are at a high risk of serious pre-

discharge morbidities. These morbidities include early and late-onset neonatal sepsis (LONS), necrotising enterocolitis (NEC), poor somatic growth besides intraventricular haemorrhage, periventricular leukomalacia, retinopathy of prematurity, patent ductus arteriosus and chronic lung disease. There is evidence of microbial intra-uterine invasion in 25% to 40% of preterm births and 7% to 12% of preterm labour with intact membranes.<sup>4</sup> The polymerase chain reaction (PCR) technique demonstrates the prevalence of microbial invasion of amniotic fluid to be 30% to 50% higher than conventional culture-based method. The amniotic fluid of preterm births can be colonised by the ascending vaginal microbes even if the amniotic membrane is intact and from placenta microbiome linked to the hypothesised haematogenous spread from maternal oral microbiome.<sup>5</sup> Although numerous studies support the notion that intestinal bacteria play an essential role in the pathogenesis of NEC, EONS and LONS, there has been no single bacterial species consistently identified as the causative agent. Acquisition of Group B Streptococcus (GBS) and *E. coli* during the birth process has been identified as the primary cause of EONS within 3 days postpartum among preterm infants. Thus, the pattern and magnitude of the bacterial gut disruption is an active ongoing research subject in preterm infants with adverse outcomes.<sup>6-8</sup>

The aim of our study was to know the incidence of colonization of the preterm neonates' gastric aspiration and to co-relate it with the various neonatal, maternal and environmental risk factors.

## METHODOLOGY

This was a prospective, observational study conducted in 100 pre-term newborns delivered at a teaching hospital of western Gujarat between October 2019 and September 2021. The study commenced after approval from institutional ethical committee. Informed consent was taken from all participants before inclusion in the study. The sample size was calculated using following formula:  $n = (Za/2)^2 + (PQ) / E^2$ . By rounding off, final sample size was 100.

All pre-term newborns, who did not have any clinical features for sepsis were included in our study. We excluded neonates who had received any antibiotic prior to collection of the first gastric aspirate sample by nasogastric feeding tube for culture, out-born neonates who were received in the institute after 24 hours of life, whose second sample could not be obtained either due to early discharge from the hospital or death of the neonate. Gestation age, sex, birth weight, obstetric history, detailed perinatal history including APGAR scores and detailed maternal history were taken. General & systemic examination was done. Gastric aspirate at 24 hours & 72 hours of life was sent for culture & sensitivity. All preterm neonates were followed for 4 weeks to see the development of neonatal sepsis. The recorded data was entered in Microsoft Excel sheet and then exported to data editor page of SPSS version 21 (SPSS Inc., Chicago, Illinois, USA). For all tests, confidence level and level of significance were set at 95% and 5% respectively.

## RESULTS

100 preterm new-borns who did not have any clinical features for sepsis, were included in our study. Mean age of mothers was  $23.99 \pm 2.11$  years with 78% mothers in the age range of 20 to 25 years. Out of the 100 cases, 78% were primi-para and 22% were multi-para. Mode of delivery was normal vaginal delivery (NVD) in 19% cases & lower segment caesarean section (LSCS) in 81% cases. As per gestation age of newborns, 30% were below 32 weeks while 70% were between 32 to 36.9 weeks. Weight of 80 newborns was below 1.5 kgs, 6 newborns were between 1.5-2.5 kgs & 14 newborns were above 2.5 kgs. Mean gestation age & weight was  $32.57 \pm 1.71$  weeks and  $1.26 \pm 0.16$  kgs respectively (Table-1).

**Table-1: Demographic profile of patients**

Characteristics		Number	Mean $\pm$ SD
Mother's age (years)	20-25	78	23.99 $\pm$ 2.11
	26-30	22	
	> 30	0	
Parity	Primi-para	78	
	Multi-para	22	
Mode of delivery	NVD	19	
	LSCS	81	
Gestation age (weeks)	< 32	30	32.57 $\pm$ 1.71
	32-36.9	70	
Weight of newborns (kilogram)	< 1.5	80	1.26 $\pm$ 0.16
	1.5-2.5	6	
	> 2.5	14	

In our study the most common organisms isolated were enterococcus (41 & 25), followed by bacteroides (26 & 17) and staphylococcus (14 & 8) in 24 hours & 72 hours of gastric aspirate samples respectively (Table-2).

**Table-2: Isolated Micro-organism in preterm neonates' gastric aspirate samples**

Isolated micro-organism	1 <sup>st</sup> gastric aspirate culture (at 24 hours of life)	2 <sup>nd</sup> gastric aspirate culture (at 72 hours of life)
Bacteroides	26	17
Bifidobacterium	2	1
E. Coli	8	5
Enterococcus	41	25
Staphylococcus	14	8
Not Found	9	44
Total	100	100

No significant association was observed between organism isolated in neonate gastric aspirates and age of mother ( $p=0.15$ ), parity of mother ( $p=0.67$ ) or mode of delivery ( $p=1.0$ ). On assessing the maternal factors, multiple ante-natal per-vaginal (PV) examinations was a significant factor responsible for colonization of the pre term neonatal gut ( $p < 0.05$ ). A significant association was also observed between gestational age (prematurity) and organisms isolated in gastric aspirates ( $p < 0.05$ ). However, no significant association was observed between gender of the baby and organisms isolated in neonate gastric aspirates ( $p=0.306$ ). Lower gestation age (GA) and birth weight were significantly associated with colonization of the pre term neonatal gut ( $p < 0.05$ ). The positive colonization was also significantly correlated to low APGAR score at 1 & 5 minutes. Significant association was observed between colonization of the pre term neonatal gut and giving of top feeds (Table-3).

**Table-3: Correlation of Isolated Organism with neonatal & maternal factors**

Neonatal & maternal factors	Organisms Isolated		Total	p-value
	No	Yes		
Multiple PV examinations	59	41	100	< 0.05
GA < 32 weeks	0	30	30	< 0.05
LBW	2	84	86	< 0.05
Out born newborns	9	63	72	0.06
APGAR at 1 min < 7	0	10	10	< 0.05
APGAR at 5 min < 7	0	8	8	< 0.05
Meconium-stained liquor	0	9	9	< 0.05
Pre-lacteal feeds	3	42	45	< 0.05

In our study, out of the 91 cases where organism was isolated, 10 cases (11%) developed neonatal sepsis as compared to 0% in cases with no organism in neonate gastric aspirate. ( $p$  value <0.05) (Table-4). Highest incidence (14.6%) of neonatal sepsis was seen with enterococci organism where six newborns with enterococci isolates in gastric aspirate developed neonatal sepsis, followed by *E. coli* isolates (12.5%). The incidence of neonatal sepsis was 7.7% and 7.1% respectively in cases where bacteroides and staphylococcus were isolated ( $p$  value 0.77) (Table 4).

## DISCUSSION

In our study, the most common organism isolated in gastric aspirate culture was enterococcus followed by bacteroides and staphylococcus. This was in accordance with study done by Wandro S et al.<sup>9</sup> While, in a study by Thaddanee R et al<sup>10</sup> most

common organism was *Klebsiella pneumonia* followed by *Pseudomonas aeruginosa*. Petersen SM et al<sup>11</sup> found that the most prevalent bacteria were *Enterococcus* spp. and *CONS*.

**Table-4: Incidence of neonatal sepsis as per type of isolated organism**

Organisms Isolated	Number of patients	Developed neonatal sepsis (N)
Bacteroids	26	2
Bifidus	2	0
<i>E. coli</i>	8	1
Enterococcus	41	6
Staphylococcus	14	1
<b>Total</b>	91	10

In the present study, no significant association was observed between age, parity of mother and organism isolated in neonate gastric aspiration. Similar results were also observed in the studies by Thaddanee R et al<sup>10</sup> and Das P et al.<sup>12</sup>

In the present study, a significant association was observed between positive neonate gastric aspirate and low gestation age of newborns ( $p<0.01$ ). La Rosa, et al<sup>13</sup> stated that the rate of a structured maturation of gut microbiome was dependent on the gestation age at birth and postnatal age. We also observed that gestation age was significantly lower among cases with presence of gut colonization. In a similar pattern we observed that, these neonates had lower birth weight. The positive colonization was also significantly correlated to low APGAR score and with giving of pre-lacteal feed or top feeds ( $p<0.05$ ). In the present study, incidence of neonatal sepsis was 11%. A significant association was observed between development of neonatal sepsis and presence of organisms in neonatal gut. Limitation of the present study is small sample size. More studies on neonatal sepsis and its relationship with the gut flora are required.

## CONCLUSIONS

This study showed a high incidence of colonization of the pre term neonate gut, with enterococcus being the most common isolate. A significant association was observed between gut colonization and development of neonatal sepsis. Significant factors for development of gut colonization included: lower gestation age, multiple PV examinations and low birth weight of the newborns. Hence, for premature neonates, gastric aspirate culture may be used as a screening test for neonatal sepsis.

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