Original Article

Colonization of the Preterm Neonatal Gut with Carbapenem-resistant *Enterobacteriaceae* and Its Association with Neonatal Sepsis and Maternal Gut Flora

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Abstract

Background: Multidrug-resistant Gram-negative neonatal sepsis is associated with high mortality and morbidity. Mucosal colonization with these organisms in hospitals may predispose neonates to septicemia. A**ims:** The aim of the study was to determine the prevalence and pattern of colonization of neonatal preterm gut with carbapenem-resistant *Enterobacteriaceae* and identify risk factors associated with colonization. **Settings and Design:** The study was a prospective observational study done in a Level 3 neonatal unit of a tertiary care hospital. **Methods:** Stool samples from preterm babies were collected soon after birth and at 1 and 3 weeks of age after consent. Maternal stool sample was collected within 48 h after the delivery. Predetermined antenatal, neonatal, and environmental risk factors were recorded. Isolation and identification of organisms was done in a standardized manner; antibiotic susceptibility was done by the Kirby–Bauer method and results interpreted according to the Clinical and Laboratory Standards Institute guidelines. **Results:** Seventy-one percent of the babies were colonized by Gram-negative bacteria (GNB) at birth, and 100% were colonized by the end of the 1st week. The organisms commonly isolated were *Escherichia coli*, *Klebsiella*, NFGNB (Nonfermenting Gram-Negative Bacilli), *Pseudomonas,* and *Enterobacter*. Sixty-eight percent of the babies were colonized with extended-spectrum beta-lactamase-producing organisms, and 5% of the babies were colonized with carbapenem-resistant organisms (CROs). In the babies who developed culture-positive sepsis, 21% had concordance of strains in the gut and blood. There was no association between maternal and neonatal colonization. **Conclusions:** The results show that neonatal gut is colonized by GNB from birth onward. However, the rate of colonization with CRO is low. An association was also observed between colonization and late-onset sepsis.

Keywords: Carbapenem-resistant organisms, colonization, intestine, preterm

Introduction

The neonatal gut becomes colonized by bacteria, starting immediately after birth, and the gut microbiome quite often reflects the maternal gut flora.[1-3] The immature intestine of the preterm infants with underdeveloped immunity, barrier function, and peristalsis makes it a potential source of infection and inflammation.[4] Although not all colonization leads to infection, the pathogenicity of the Gram-negative bacteria (GNB) and poor immune status of the preterm babies may predispose them to septicemia caused by these organisms.[1]

Carbapenem-resistant organisms (CROs) are an emerging problem in the world associated with high mortality and morbidity. There are several studies which have examined the pattern of neonatal gut colonization with GNB and have demonstrated the correlation between gut colonization and sepsis. However, there are only a few studies on the prevalence of CROs colonization in the gut of preterm babies.[5] Hence, this study was done to (i) determine neonatal and maternal colonization with these organisms, (ii) identify risk factors associated with colonization, and (iii) look for the association between colonization and late-onset sepsis.

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Methods

This was a prospective cohort study done over a period of 12 months in the neonatal unit of a tertiary care perinatal center in South India. Data were collected and entered prospectively in the given pro forma. The study was approved by the institutional research board and the ethics committee, and informed consent was obtained from the parents before recruitment. All very low birth weight (VLBW) inborn neonates < 1500 g born during the study period were eligible for the study. Sample size was calculated with the estimated prevalence of CROs at 5%, with a precision of 4% in eighty babies. We excluded babies with multiple congenital anomalies and babies critically ill at birth, who were unlikely to survive.

Once consent was obtained, the first stool that the baby passed was sent immediately to the microbiology laboratory, where it was plated onto blood agar, MacConkey agar, selenite F, and deoxycholate citrate agar. After overnight incubation at 37°C, suspected colonies were identified biochemically; their antibiotic susceptibility was done by Kirby–Bauer method and results interpreted according to the Clinical and Laboratory Standards Institute guidelines. Extended-spectrum beta-lactamase (ESBL) was defined as resistance to cephalosporins, and CRO was defined as resistance to either imipenem or meropenem. We followed up the babies up to 4 weeks of age, and subsequent stool samples were collected between 7–14 days and 21–28 days of life. Mother's stool sample was collected within 48 h of recruitment and analyzed similarly.

For molecular characterization, the whole genomic DNA was extracted from overnight colonies grown on blood agar, using the QIAamp DNA Mini Kit and the QIA cube instrument (Qiagen, Valencia, CA, USA), according to the manufacturer's instructions. Isolates were screened for the presence of the following genes encoding for carbapenemase *bla*IMP, *bla*VIM, *bla*NDM, *bla*OXA-48, and *bla*KPC.

Polymerase chain reaction (PCR) assay was performed using Qiagen master mix kit. Amplicons were visualized under ultraviolet in a 2% agarose gel containing ethidium bromide, and the controls were compared simultaneously.

All babies were treated as per the unit's protocol. If any baby developed signs of sepsis, blood culture and sepsis screen were collected and antibiotics started, as per the unit protocol. Outcome measures included the number of babies colonized with CROs, time profile of colonization, association with various risk factors, similarity between organisms isolated from the neonatal gut, and those isolated from blood culture and maternal gut.

Statistical analysis for risk factors influencing gut colonization by CROs was done by multiple logistic regression.

Results

One hundred and one babies (62 males) were included in the study. The mean birth weight was 1272.4 ± 176 g, whereas the mean gestational age was 31.6 ± 2.2 weeks. Sixty-seven (66.3%) of the babies were delivered by LSCS (Lower segment Caesarean section). The duration of rupture of membranes was more than 24 h in 10 (9.9%) deliveries and 25 (24.7%) mothers received intrapartum antibiotics. 16 (15.8%) babies required resuscitation at birth, 99 (98%) were given intravenous (IV) fluids, and 67 (66.3%) were given TPN (Total parenteral nutrition).

Prevalence of gut colonization with Gram-negative bacteria

Seventy-one percent of the babies were colonized at birth. All babies were colonized by the end of the 1st week and 97% persisted to have GNB in their stools at 3 weeks of life [Figure 1]. The most commonly isolated organisms in stool samples were *Klebsiella* (68%), *Escherichia coli* (66%), *NFGNB* (9%), *Pseudomonas* (7%), and *Enterobacter* (6%).

Sixty percent of the organisms were ESBL producers in stool culture 1 and the percentage became 67% and 76% with stool cultures 2 and 3, respectively. Seven percent of the organisms were CRO in stool cultures 1 and 2, whereas the stool culture 3 had 3% CRO [Figure 2].

E. coli and *Klebsiella* were the most common organisms found in the mother's stool with 82% and 51% cultures growing them. Ten percent organisms in maternal stools were ESBL producers. There was 90% sensitivity to cephalosporins in contrast to 32% found in the babies and 99.5% sensitivity to carbapenems. There was no association between maternal and neonatal gut colonization.

Nineteen babies (18.8%) developed blood culture-positive sepsis, out of which only 4 (21%) had the same organism in blood and stool cultures.

The risk factors associated with colonization with CRO are summarized in Table 1.

Other factors looked at were the presence of NG tube, IV fluids, TPN, and blood products administration and were not found to be significantly associated with colonization.

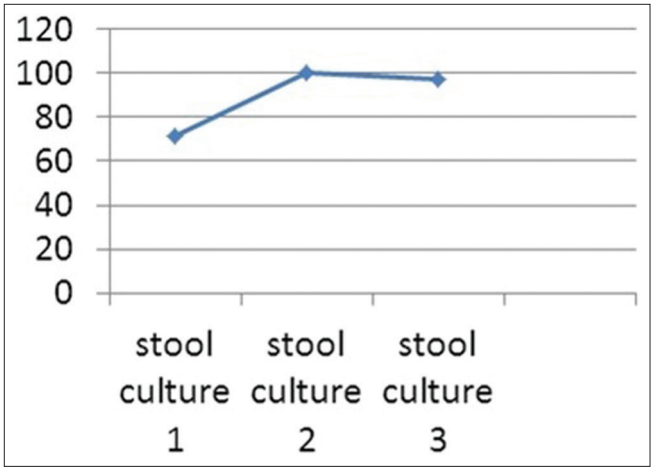

Figure 1: Prevalence of gut colonization with Gram-negative bacteria at birth (stool culture 1), 1 (stool culture 2), and 3 (stool culture 3) weeks

Figure 2: Percentage of extended-spectrum beta-lactamase and carbapenem-resistant organism colonization at birth, 1, and 3 weeks

Table 1: Factors associated with gut colonization by carbapenem-resistant organisms

Factor influencing	OR	95% CI	*P*
Umbilical catheter	19.8	1.8-215	0.014
Resuscitation at birth	7.14	1.4-35.2	0.016
IMV	7.88	1.89-32.9	0.005
CPAP	3.6	0.94-13.7	0.06
ANC <500	30.28	2.9-310	0.004
Thrombocytopenia	9.6	22-41.2	0.002
Leukopenia	7.14	1.44-35.22	0.016

CI: Confidence interval, OR: Odds ratio,IMV: Intermittent Mandatory Ventilation, CPAP: Continous Positive Airway Pressure, ANC: Absolute Neutrophil Count

Multiplex PCR for the detection of carbapenem-resistant genes was done on four of the seven CRO isolates and showed the presence of New Delhi Metallo-beta-lactamase gene in three of them [Figure 3].

Discussion

Sepsis due to CROs is an emerging health problem and is associated with the increased morbidity and mortality of hospitalized patients. In the DeNIS study that looked at neonatal sepsis across three neonatal intensive care units (NICUs) in New Delhi, 35% of *Klebsiella* and 15% of *E. coli* were carbepenem resistant with a case fatality rate of >50%.[6] Fecal carriage is of particular importance as infections caused by *Enterobacteriaceae* are common. Colonization in preterm babies may be associated with diseases such as necrotizing enterocolitis and late-onset sepsis. As these organisms are resistant to most antibiotics, they are difficult to treat and associated with a high mortality.

Colonization of the newborn gastrointestinal tract is affected by maternal and environmental sources. The pattern of colonization is different in premature infants in an intensive care setting, when compared to healthy, term breastfed infants.[7] Preterm infants have a premature intestine with underdeveloped peristalsis, barrier function, and immunity, which makes it a source of infection and inflammation.[4,8,9] Concordance of colonizing and invasive strains has been

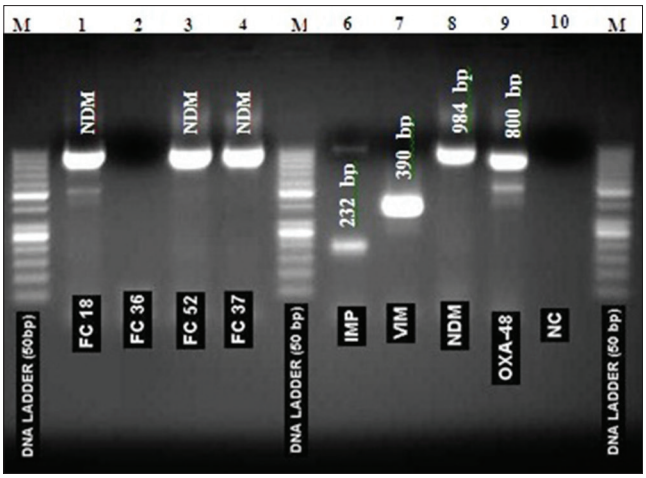

Figure 3: Multiplex polymerase chain reaction for the detection of carbapenem-resistant genes. Lanes 1–4: *Klebsiella* spp. isolated from feces sample. Lanes 6–9: Positives control for IMP, VIM, NDM, and OXA-48 genes. Lanes M: Molecular weight marker (50 bp size marker). Lane 10: Negative control (no template DNA added)

demonstrated previously.[10] A study done in New York demonstrated 98% concordance between surveillance cultures caused by the same strain and same gentamicin susceptibility profile and later bloodstream infections.[11]

In our study, we found preterm gut colonization rates of 71%, 100%, and 97% at birth, week 1, and week 3 of life. Studies done in the northern parts of the country have shown similar colonization rates.[1,12] This is higher than colonization rates in the West.[13]

The most common organisms isolated from the stool cultures in our study were *Klebsiella* and *E. coli*. These organisms were consistently found across all cultures. A recent study done in the USA on 32 VLBW infants has recorded relative abundance of *Enterobacteriaceae, Enterococcus, Staphylococcus,* and *Bacteroides* organisms.[14]

During this study, the overall resistance to ESBLwas 68% and that to CRO was 5%. A recent study done in the northern part of India has reported carbapenem-resistant *Enterobacteriaceae* gut colonization in neonates as 8.7%,[5] whereas a study done in Germany has shown ESBL *Enterobacteriaceae* colonization rate in preterm infants to be 5.7%.[15] In an Italian study, the ESBL colonization rate was found to be 27.1%,[16] whereas a study done in Cambodia showed colonization by a CRO in 7.5% of cases.[17]

During our study, 19 (18.8%) babies had positive blood cultures. Nine (47.3%) out of the nineteen babies had the same organism causing sepsis and colonizing the gut, but only 4 (21%) had the same sensitivity pattern, suggesting that the source for these was from the neonatal gut. It was interesting to note that the two babies with CRO sepsis had CRO in stools also. Concordance of colonizing and invasive strains has been demonstrated in other studies.[17-19]

Mothers' stool cultures were also analyzed for the presence of GNB in our study. GNB could be isolated from all

except two samples. The common organisms here also are *E. coli*, *Klebsiella,* and *Enterobacter*. In a study done in Sri Lanka, it was found that mothers were colonized with *Enterobacteriaceae* in 18.8% of the cases.[20] The German study mentioned earlier has shown the prevalence of ESBL *Enterobacteriaceae* colonization among mothers as 11.1%.[15]

However, what we found was that the sensitivity pattern of the mothers' gut flora was strikingly different from that of the babies.

Conclusions

The gut of preterm babies is colonized with potentially pathogenic bacteria, starting almost immediately after birth, although the rate of colonization with CROs is low.

In cases of culture-positive sepsis, concordance of invasive and colonizing strains was found in 21% of the cases. No correlation existed between maternal and neonatal gut flora, suggesting that the environment contributes to the gut colonization of preterm NICU babies.

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Conflicts of interest

There are no conflicts of interest.

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