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'Necrotizing Enterocolitis' In Premature Neonates - The Putative Role of Early Gastrointestinal Colonization

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Abstract:

Introduction: *Necrotizing enterocolitis (NEC) is a gastrointestinal emergency and a major cause of morbidity in the neonatal intensive care unit (NICU).We studied the risk factors, pattern and type of microorganisms colonizing the gastrointestinal tract of neonates with NEC, their antibiotic susceptibility and its role in NEC.*

Material and methods: *40 neonates were included in our prospective case control study over 15 months, 18 with NEC and 22 with no NEC. Risk factors for NEC were assessed. Oral and rectal swabs collected on day 1, day 3 & day 7 and processed. Antibiotic susceptibility testing was performed on the isolates based on CLSI guidelines.*

Results and conclusion: *Of neonates with NEC 100 % were preterm, delivered by lower segment Caesarean section and on parenteral nutrition, 44 % were of low birth weight, 56 % of very low birth weight, 89 % were given formula feeds and none were colonized on day 1. Klebsiella sp., Enterobacter sp., Acinetobacter sp. and S. aureus were isolated relatively more from neonates with NEC. Resistance to antibiotics used for treatment- ampicillin (52-57%), gentamicin (31-82%), cefotaxime (43-80%) for NEC was found. Risk factor association and change in antibiotic resistance prevalence was statistically significant. Delayed colonization, also pathogenic strains were seen to be associated with NEC in premature infants in the NICU. Acquisition of these nosocomial and resistant pathogens as flora in the NICU is an issue of concern.*

Keywords: ***Necrotizing enterocolitis, neonates, NICU, colonization, gastrointestinal flora.***

Introduction:

Necrotizing enterocolitis (NEC) is a gastrointestinal emergency and a major cause of morbidity among neonates admitted in the neonatal intensive care unit (NICU)[1]. 90 % of those who develop NEC are born preterm requiring critical intervention and support, the risk being inversely related to their birth weight and gestational age[1-3].

Despite decades of research, the pathogenesis of necrotizing enterocolitis in preterm neonates is unclear. The sequence of events leading to NEC is complex and multifactorial[1]. The three major risk factors implicated are prematurity of intestine, enteral feeding and microbial colonization (Figure 1)[2]. Colonization of the sterile gastrointestinal tract of the neonates begins right from the day of birth[4]. This complex process is influenced by microbial and host interactions, and by various internal and external factors[4, 5].

The microbial flora in the intestine of hospitalized premature neonates is markedly different than the intestinal microbial environment found in full-term, breast-fed neonates[6]. In the premature neonates, immaturity of the intestinal epithelial barrier and neonatal mucosal immune system predisposes them to bacterial invasion and infection triggering the pathogenic sequence in NEC. The immature intestinal barrier lacks several key protective mechanisms that normally prevents invasion by luminal bacterial flora. The compromised gut barrier together with an altered bacterial flora in premature infants therefore stimulates the production of pro-inflammatory cytokines further compromising intestinal defence mechanisms. This imbalance between cell injury and repair leads to a vicious cycle of bacterial invasion, immune activation, uncontrolled inflammation, and gut barrier failure thus leading to necrotizing enterocolitis. The cascade of inflammation may even progress to intestinal necrosis, perforation with sepsis and death (Figure 2)[1-4]. Before we develop strategies to prevent and treat NEC in the NICUs we need to understand the causal role of early microbial colonization of the sterile gastrointestinal tract of the neonates admitted in the NICU.

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We intended to study the risk factors associated with developing NEC, the pattern of gastrointestinal colonization in neonates with NEC, the association of the gastrointestinal isolates with NEC and the antibiotic resistance in these isolates, also their role in NEC.

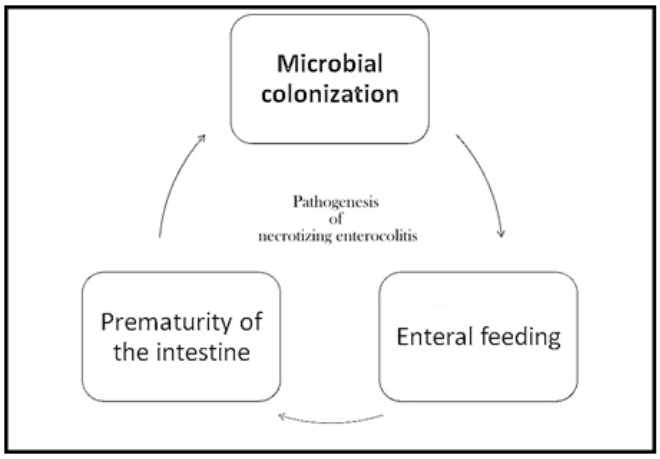

Figure 1: Factors contributing to the development of necrotizing enterocolitis

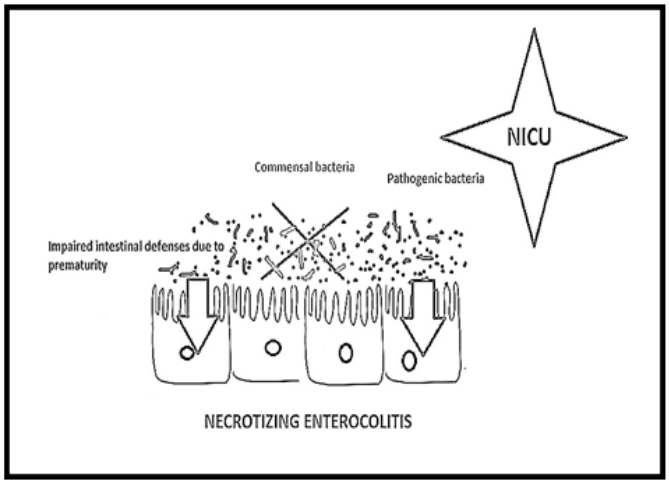

Figure 2: Mechanism of NEC as caused by colonizing pathogenic bacteria in the NICU

Materials and Methods:

Study design and settings: We conducted a prospective case control study between the period of October 2011- January 2013 which included 40 neonates from the NICU of Lady Goschen Hospital, Mangalore.

Study population: After obtaining the institutional ethics committee clearance and a written informed consent obtained from parents / guardians we recruited 40 neonates in our study. We included neonates admitted in NICU for a minimum period of 7 days. Neonates with congenital malformations of gastrointestinal tract, those who had gastrointestinal surgeries, those who were admitted to the NICU from the wards and those born outside the hospital were excluded from our study.

Clinical data: A detailed clinical history regarding mode of delivery, gestational age, birth weight, APGAR score, feeding and nutrition, parenteral feeding, indwelling devices, antimicrobial therapy was collected. Based on the modified Bell's staging criteria for clinical diagnosis of NEC which is based on systemic, intestinal and radiological signs, neonates were categorized into 'Suspected' (Stage 1), 'Definite' (Stage 2) and 'Advanced'(Stage 3) cases of NEC[1, 4, 17].

Sample collection and processing: Oral and rectal swabs were collected on day 1 of birth, day 3 and day 7 from the neonates. The samples were immediately transported to the laboratory without any delay. Gram stain was done and the samples were plated on chocolate agar medium and Mac-Conkey agar medium, the culture plates incubated at 370 Celsius for 18-24 hours. Depending on the growth pattern, morphology of colonies and standard biochemical reactions all the isolates were identified. The amount of growth was noted with each successive sample of the neonate (semi quantitative method). Antibiotic susceptibility testing was performed on Mueller Hinton agar by Kirby Bauer's disk diffusion method based on CLSI guidelines[19, 20]. The antibiotics for testing chosen based on isolates and the antibiotic policy of the NICU in our hospital.

Data analysis: The collected data and microbiological results was analysed using SPSS version 11.5 software, appropriate tests were used and p value < 0.05 was considered as significant.

Results:

Table 1: Comparison of risk factors for NEC among the NICU neonates

RISK FACTOR	NEONATES WITH NEC n=18	NEONATES WITHOUT NEC n=22	NEONATES WITH NEC n=18	NEONATES WITHOUT NEC n=22
MODE OF DELIVERY	NVD[1]	NVD[1]	LSCS[2]	LSCS[2]
	-	32 %	100 %	68 %
GESTATIONAL AGE	TERM	TERM	PRETERM	PRETERM
	-	64 %	100 %	36 %

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NUTRITION	ONLY BREAST FED	ONLY BREAST FED	BREAST FED + FORMULA FED	BREAST FED + FORMULA FED
	2 %	64 %	88 %	36 %
PARENTERAL FEED	NO PARENTERAL FEEDING	NO PARENTERAL FEEDING	PARENTERAL FEEDING	PARENTERAL FEEDING
	-	50 %	100 %	50 %

BIRTH WEIGHT	NEONATES WITH NEC n=18			NEONATES WITHOUT NEC n=22		
	NORMAL	LBW[3]	VLBW[4]	NORMAL	LBW[3]	VLBW[4]
	-	45 %	55 %	50 %	23 %	27 %

(NVD=normal vaginal delivery, LSCS= lower segment caesarean section, LBW = low birth weight, VLBW = very low birth weight)

Table 2: Statistical analysis of risk factors for colonization as analysed by Fisher's exact test

Risk factor	P value by Fisher's exact test
Mode of delivery	0.0109
Birth weight	0.003
Gestational age	< 0.001
Place of admission	<0.001
Formula feeding	<0.001
Parenteral nutrition	0.003

(P < 0.05 = significant)

Table 3: Colonization rate in neonates with NEC and no NEC in the NICU

DAY OF SWAB COLLECTION	ORAL		RECTAL	
	No NEC n = 22	NEC n = 18	No NEC n = 22	NEC n = 18
DAY 1	64 % (14)	- (0)	54 % (12)	- (0)
DAY 3	100 % (22)	78 % (14)	100% (22)	78 % (14)
DAY 7	100 % (22)	100 % (18)	100% (22)	100% (18)

Table 4: Organisms isolated from oral swabs from the neonates

ISOLATE	Neonates with NEC n= 18			Neonates with no NEC n=22		
	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7
Streptococcus viridans group	0	8	9	7	16	18
S. aureus	0	6	9	5	9	12
Enterococcus spp.	0	0	1	3	4	4
E.coli	0	2	2	1	3	3
Klebsiella spp.	0	4	5	0	0	1
Acinetobacter spp.	0	2	2	2	0	0
Enterobacter spp.	0	1	3	1	1	1
Pseudomonas spp.	0	0	0	1	1	1

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Table 5: Organisms isolated from rectal swabs from the neonates

ISOLATE	Neonates with NEC n= 18			Neonates with no NEC n=22		
	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7
S. aureus	0	7	12	2	8	12
Enterococcus spp.	0	4	7	2	6	6
E.coli	0	2	8	3	4	11
Klebsiella spp.	0	7	12	1	4	7
Acinetobacter spp.	0	2	4	0	0	0
Enterobacter spp.	0	2	8	1	1	1

Of the 40 neonates included in our study based on the clinical criteria for diagnosis of NEC, 18 of them with were considered as cases of NEC and the other 22 neonates with no clinical evidence served as the control group of our study. 72% of these neonates (29/40) were born preterm, 82% (33/40) of them were delivered by cesarean section, 32% (13/40) and 40% (16/40) were of low birth weight (< 2,500 g) and of very low birth weight (< 1,500 g) respectively. The clinical profile of these neonates with and without NEC is summarized in Figure 3. We compared the following risk factors between the two groups: mode of delivery, gestational age, birth weight, and type of feeding and parenteral feeding. We found that 100% of neonates with NEC were born preterm and delivered by cesarean section, 44% (8/18) and 56% (10/18) were of low birth weight and of very low birth weight respectively. All the cases of NEC were on parenteral feeding once diagnosed, 89 %(16/18) were given one or the form of supplementary feeding like formula feeds apart from breast feeding during the weaning period from parenteral nutrition with the tapering of their treatment (Table 1).

For the statistical analysis, Fisher's exact test was used to compare the risk factors in these two groups and the results were found to be statistically significant (Table 2). Of interest was the rate of colonization in these two groups, none of the neonates with NEC showed oral and rectal colonization on day 1 of birth, 78% of them were colonized on day 3 and 100 % on day 7. But colonization in neonates without NEC was seen on day 1 which was early compared to neonates with NEC (Table 3).

When we considered the spectrum of microorganisms colonizing the gastrointestinal tract of the neonates with NEC, we found that non Escherichia coli enterobacteria like Klebsiella sp., Enterobacter sp. and Acinetobacter sp. were isolated in comparatively higher numbers from both oral and rectal swabs from this group (Table 4 & 5).

For the antibiotics tested for susceptibility in all isolates, the change or increase in resistance prevalence from day 1 to day 7 was analyzed by Kruskal Wallis test (a non-parametric test) with degree of frequency (d.f) = 2 & confidence interval(CI) = 95%, and p value < 0.05 was considered statistically significant. The results indicated an increased prevalence of antibiotic resistant flora by day 7 in neonates with NEC (Figure 4) predominantly to ampicillin, gentamicin, cefotaxime, ciprofloxacin and piperacillin. All these neonates with NEC were on prophylactic antibiotic therapy with ampicillin, gentamicin, cefotaxime or metronidazole. Methicillin resistant Staphylococcus aureus (MRSA) and extended spectrum beta lactamases (ESBL) producing E.coli and Klebsiella sp. were also isolated in higher numbers from these neonates with NEC over their course of their stay in the NICU (Figure 5 & 6).

Of the 18 neonates with NEC, 7 were suspected, 6 were of definite diagnosis and 5 were in advanced stage. All the cases were kept nil per oral (NPO) and given parenteral feed. Prophylactic antibiotics were given and their vitals monitored. 5 neonates succumbed to NEC; a mortality rate of 28 % was seen in our study despite medical and surgical interventions.

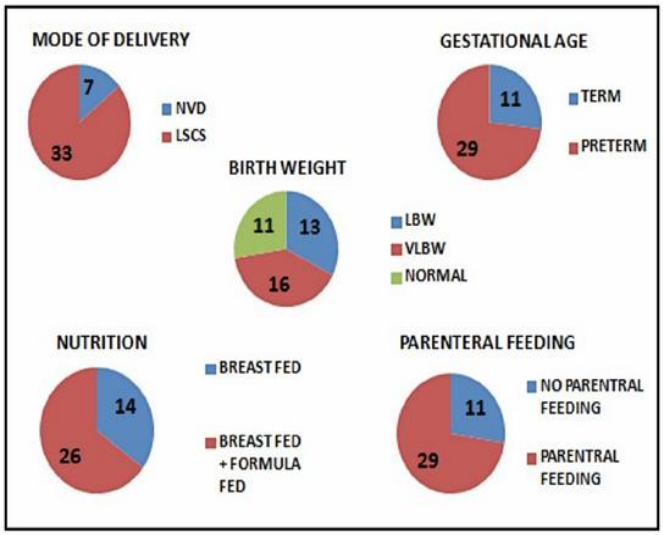

Figure 3: Clinical profile of neonates admitted in the NICU, n=40 (NVD=normal vaginal delivery, LSCS= lower segment caesarean section, LBW = low birth weight, VLBW = very low birth weight)

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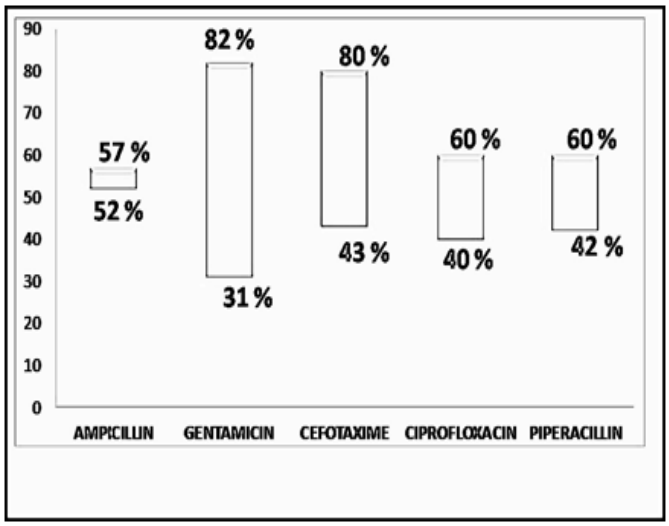

Figure 4: Antibiotic resistance range (minimum to maximum) of the Enterobacterial isolates as analyzed by the Kruskal Wallis test to the significant antibiotics used for prophylaxis and treatment of neonates with NEC in the NICU

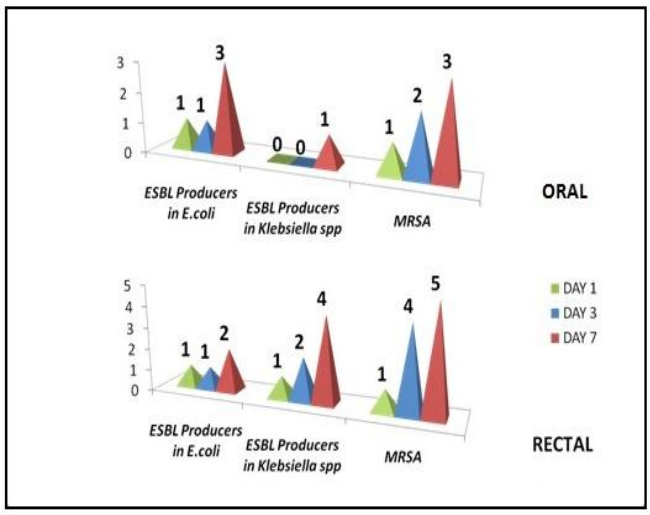

Figure 5: ESBL producers and MRSA isolates in neonates with no NEC

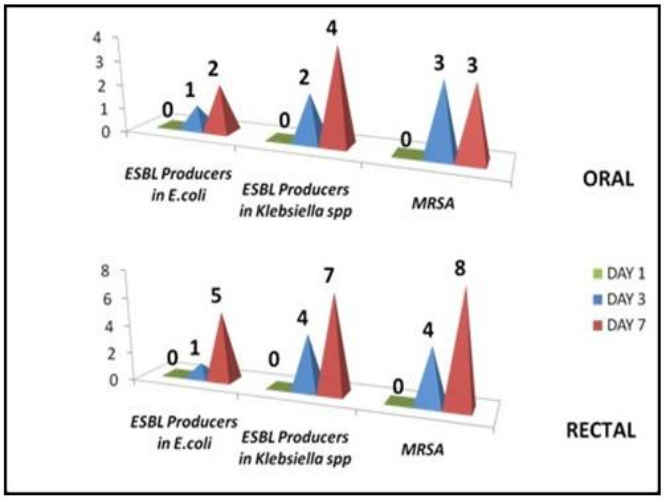

Figure 6: ESBL producers and MRSA isolates in neonates with NEC

Discussion:

Delayed gastrointestinal colonization with commensals in neonates as seen in our study is an important risk factor for developing NEC; as also reported by Fanaro et al and Hunter et al[1,5]. The degree of bacterial exposure during the neonatal period has a profound influence on this critical process. Obstetric and hygiene practices which aim at reducing the spread of pathogenic bacteria especially in the intensive care unit have synergized to result in a delayed or absent colonization. Non E.coli enterobacteria like Klebsiella sp., Enterobacter sp. along with Staphylococcus sp. and Clostridia sp. are commonly implicated in NEC in previous studies[1,4]. In our study we found isolates of Klebsiella sp., Enterobacter sp. and Acinetobacter sp. along with S. aureus in higher numbers from neonates with NEC which is in agreement. Premature infants in the NICU are probably more exposed to the nosocomial flora and therefore are prone to be colonized by these potentially pathogenic bacteria.[1-18].

The clinical factors contributing to gastrointestinal colonization of the neonates are characteristically influential and mutually dependent. The main contributing factors for NEC are admission to the NICU, preterm, low birth weight, formula feeds and parenteral feeding[1,5,16]. The initiation of parenteral nutrition in this group of neonates especially in the NICU comes with problems of bypassing the normal gastrointestinal tract especially when the crucial process of colonization is happening. This can have immediate and long term effects on the health of the neonate in the NICU. All these risk factors were observed in the neonates with NEC in our study. In addition, the significant change in the antibiotic susceptibility of the gastrointestinal flora especially to the antibiotics used for treatment as seen in our study adds to the problem of poor prognosis. Clusters of nosocomial gram negative bacterial infections are known to disseminate in a NICU setting, and strains which cause them are known to have multi drug resistance which may have occurred due to use of antibiotics which create unfavourable ecological impact by enhancing their spread[7, 22-25, 28].

The limitations of such studies include extreme subject variability and multiple factors which affect gastrointestinal colonization which are clustered and inter dependent. The effect of individual determinants can be distinguished only if a large population over a period of time is studied. Also the microbial composition of the upper part of the gastrointestinal tract largely remains unknown due to constraints of sampling techniques[8].

Microbial colonization of the gastro intestinal tract has a greater role in health and disease than we are aware of today. In neonatal necrotizing enterocolitis, its modulation and prevention has further scope for research[9]. Horizontal

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transfer of microorganisms can occur between the neonates through the hands of medical personnel. The very low compliance with hand hygiene rules in most hospitals especially in the neonatal wards and the NICUs is a major obstacle to prevent the cross-transmission of resistant and susceptible micro-organism among neonates which are responsible for NEC[6, 24]. Simple measures to decontaminate the hands before and after handling the neonates like alcohol hand rubs or proper use of gloves are very helpful in the NICUs. Several other beneficial factors such as the early initiation of breast feeding, the reduction of unnecessary antibiotic exposure and a decreased length of therapy for some nosocomial infections could be a valuable attempt to influence the gastrointestinal flora and to promote faecal microbial diversity in these vulnerable neonates, and may thus decrease the possibility of NEC in them.

Conclusion:

Our key message is that in the current era of antibiotic resistance, the first life event- birth and the first place of admission- NICU should ideally be the first area of concern: to prevent life threatening infections like NEC, to limit spread of pathogenic and antibiotic resistant strains and to establish rational antibiotic usage in the NICUs.

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Conflict of interest: None

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