

Study of Factors associated with pathological and aerobic microbial colonization of Neonatal Gastrointestinal System – A hospital based Case control study

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Introduction: The rate and extent of neonatal gastrointestinal tract colonization depends on various perinatal and neonatal factors. Colonization of gastrointestinal tract (GIT) after birth could be delayed because of prematurity, caesarean delivery or total parental nutrition. The ill and preterm neonates in NICU acquire intestinal flora that differ from that of healthy newborn. **Objective:** Hence we aim to study the factors associated with pathological colonization of aerobic microbial flora in neonates and find pattern of pathogenic aerobic microbial colonization of neonates. **Methodology:** The study was done on 94 neonates in a tertiary teaching hospital NICU , 51 cases with pathological colonization ,43 control with normal colonization. Data collected using Performa, Oral and rectal swabs taken **Results:** 68 neonates who received antibiotics 46 (90.2%) were cases 22(51.3%) were controls. *Staphylococcus aureus* was predominant suggesting influence of maternal colonization. Cases were more in babies born through LSCS and in babies whose mother had received antibiotics **Conclusion:** Of all factors influencing establishment of pathological microbiota in neonatal GIT which are interdependent , neonatal exposure to antibiotics was the most significant . Emergence of MRSA , ESBL are of particular concern as these are a constant threat as source of health care associated infection.

Introduction: The postnatal period of a new being offers a great opportunity to study, from the very beginning, the formation of a new ecosystem: the micro flora of the human gut. At birth the fetus is sterile, and the first encounter with the microbial world begins during delivery¹. During the birth process and rapidly thereafter, microbes from the mother and surrounding environment colonize the gastrointestinal tract of the infants until a dense, complex micro biota develops². The rate and extent of neonatal gastrointestinal tract colonization depend on various perinatal and neonatal factors, such as gestational age, mode of delivery, place of hospitalization (nursery versus neonatal intensive care unit), type and mode of feeding and anti-bacterial treatment. However, colonization of the gastrointestinal tract after birth could be delayed because of prematurity, caesarean delivery, or total parenteral nutrition³. The bacteria colonizing the infant gut during the first days of life originate mainly from the mother and the environment. In early life, one of the first major determinants of the gut microbiota is the mode of delivery. Vaginally born infants are colonized at first by faecal and vaginal bacteria of the mother, whereas infants born through caesarean section are exposed initially to bacteria originating from the hospital environment and health care workers⁴. If infants are separated from their mothers for long periods after birth, the environment becomes an important source of colonising bacteria⁵. The present study will focus on the factors determining the development of the pathogenic aerobic microbial flora in the gastrointestinal tract of the neonates admitted in the NICUs and wards.

The study results will help in better implementation of existing control measures and development of antibiotic policies for NICUs in our hospitals.

Study Setting: The study was conducted in the neonatal intensive care units and wards of Government Lady Goshen Hospital, Mangalore being hospital based case control study to assess the factors associated with development of pathogenic microbial gut flora, risk factors and antibiotic susceptibility in neonates admitted in the wards and the NICUs.

Study Population: Neonates admitted in the wards and NICUs was included in the study, about 94 neonates with 51 cases and 43 controls.

- Neonates with pathological colonization were considered as cases and those with normal colonization was considered as controls
- Multi drug resistant organisms, Gram negative organism other than *enterobacteriaceae-Pseudomonas, Acenatobactor*, True pathogen-*Shigella, Salmonella* where considered as pathological colonization

Neonates with congenital malformations of gastrointestinal tract and gastrointestinal surgeries were excluded from the study. After getting approval from the institutional ethics committee and informed consent from parents data was collected using a proforma

Data was analyzed using SPSS version 11.5. The statistical test Unpaired T test and Mann Whitney U test was used and p value < 0.05 was considered significant. Oral and rectal swabs were taken on Day 1, Day 3 and Day 7 or day of discharge (which ever is earlier) of the neonates. All samples were collected under complete aseptic precautions and then forwarded to laboratory immediately for processing

Sepsis diagnosed on basis of positive sepsis screen- described by Hewitt et al⁶. as any two of the below mentioned being positive

1. Band: Neutrophil ratio>0.2
2. Leukocyte count<5000/mm³
3. CRP>0.8mg/100ml
4. Micro ESR>15mm/hr
5. Haptoglobin>25mg/100ml)

Results: The hospital based case control study was done over a span of one year in the district Maternity Hospital of Mangalore and 94 neonates were studied on day 3 of life, with 51 cases- pathological colonization and 43 controls – physiological colonization. Of 68 neonates who had received antibiotic, 46 (90.2%) were cases and 22 (51.2%) were controls(Table 1). Out of 26 neonates that had not received antibiotic, only 5 were cases and 21 were controls. Out of 22 control who had received antibiotic, majority were exclusively breast feed and was in NICU, with no difference in terms of gestational age. It was found that out of 60 neonates with sepsis, the rate of sepsis was significantly more (72.5%, n=37) in cases than in controls (53.5%, n=23) with a p value=0.004 which was significant. The neonates who were controls who had sepsis, 55% were from ward, majority of them were on formula feeds. Of the cases who did not have sepsis (27,5%, n=14), 85% were from ward, all were on predominantly formula feeds with no differences based on gestation.Of all the microbial growths in cases and controls irrespective of mode of delivery, feeding practices, place of admission and gestion *Staphylococcus aureus* predominated followed by *Escherichia coli* and *Klebsiela pneumonia*(Table 2). The predominance of *Staphylococcus aureus* in controls suggest influence from maternal colonisation.

Considering the pattern of colonisation of pathological organisms, MRSA predominated with 52.5%, followed by ESBL 43%. (Table 2) Significant number of ESBL producers were E.Coli and the rest Klebsiella pneumonia, most of them were in the ward and was on formula feeds. It was noted that cases were more in the wards than in NICU irrespective of other influencing factors ($p=0.679$, not significant), possible explanation is the prevalence of MRSA that needs to be confirmed by cultures from the immediate environment the baby is in (health care personnel, bed, hospital vessels, maternal factors). Cases were more in babies born through LSCS than vaginal route ($p=0.535$, not significant), possible reason being immediate contact with hospital environment, health care personnel and separation from mother. Majority of cases were found in term neonates ($p=0.516$, not significant), most of them were in the ward with predominantly formula feeds. Cases were found to be breast fed babies ($p=0.509$, not significant), majority of them were from the ward with no difference in terms of gestational age. (Table 3) It was found that cases were more in babies whose mothers had received antibiotic; most of them were on formula feeds and from ward.

Discussion: The postnatal period of a new human being offers a great opportunity to study, from the very beginning, the formation of a new ecosystem: the micro flora of the human gut. At birth the fetus is sterile, and the first encounter with the microbial world begins during delivery⁷. During the birth process and rapidly thereafter, microbes from the mother and surrounding environment colonize the gastrointestinal tract of the infant until a dense, complex microbiota develops. In this study we have found that among the various factors influencing the establishment of pathological microbiota in the neonatal gastro intestinal system which are interdependent, neonatal exposure to antibiotics was the most significant (Table 1). The predominance of *S. aureus* in the controls suggests maternal colonisation. The emergence of MRSA, ESBL are of particular concern as these are a constant threat as a source of epidemic or source of hospital acquired infection. Our studies result supports Lindberg E et found that many Swedish infants carry *Staphylococcus aureus* in their intestinal micro flora. The source of this colonization of infantile intestinal *S. aureus* was strongly associated with maternal colonisation *S. aureus* carriage. These suggest that *S. aureus* on parental skin establish readily in the infantile gut, perhaps due to poor competition from other gut bacteria⁸. Lorenzo et al found that naturally delivered babies experienced a period of 2-3 d in which within the gut belong to aerobic species such as Enterobacteriaceae, streptococci, and staphylococci. These bacteria, arriving from the external environment, belong to species with a pathogenic potential, and therefore, it might seem that they would not be the best choice for the health of neonates. Skin-derived staphylococci are becoming more abundant than fecal Enterobacteriaceae.

Conclusions: Of all the factors influencing the establishment of pathological microbiota in the neonatal gastro intestinal system which is interdependent, neonatal exposure to antibiotics was the most significant. The predominance of *S. aureus* in the controls suggests maternal colonisation. The emergence of MRSA, ESBL are of particular concern as we have seen that the sepsis rate was more in cases suggesting that these are a constant threat as a source of epidemic or source of hospital acquired infection. Infection control has an important role in hospitals and there is a pressing need to develop guidelines and preventive measures. Exposure to non beneficial (pathogenic) microorganism and antibiotic early in life may result in immune dysregulation, aberrant barrier functions and altered gut function, that in susceptible individuals may lead to some diseases later in life

Table 1: Proportion of cases based on antibiotic usage.

ANTIBIOTIC USAGE	CASES N (%)	CONTROLS N (%)	TOTAL N (%)	P-value
No	5 (9.8)	21 (48.8)	26 (27.7)	0.001
Yes	46 (90.2)	22 (51.2)	68 (72.3)	
TOTAL	51	43	94	

Table 2: Pattern of colonisation of pathogenic organism

PATHOGENIC ORGANISM	FREQUENCY	PERCENT(%)
ESBL	35	43
HLAR	3	3.4
MRSA	43	52.5
VRE	1	1.5
TOTAL	82	100

Table 3: Proportion of cases based on type of feeding

TYPE OF FEEDING	CASES N (%)	CONTROLS N (%)	TOTAL N (%)	P-value
Breast fed	33(64.7)	31(72.1)	64(68.1)	0.509
Breast fed +Formula fed	18(35.3)	12(27.9)	30(31.9)	
TOTAL	51	43	94	

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