Mycobacterium species on the cutaneous microbiome of very preterm neonates

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Short Report

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Abstract

The neonatal skin microbiome consists of all the genomes and genetic products of microorganisms harbouring on the skin of babies. Host and the microbiota develop a harmonious environment resulting in symbiosis. Any disruption of this environment could lead to pathological disease. We conducted this study to understand the neonatal skin microbiome of very preterm neonates admitted to NICU at a tertiary health care setting before and after Kangaroo Mother Care. Next Generation sequencing showed relative abundance for Mycobacterium tuberculosis in 83.33% & 66.67% (p0.285) and Mycobacteroides abscessus in 100% & 93.33%(p0.303) of the very preterm neonates on the skin microbiome before and after KMC respectively. The mere presence of Mycobcaterium species as commensals or as potential pathogens, is alarming due to the risk of early exposure and incidence of tuberculosis right from birth. These findings, in our view, are the first findings to be established in such a setting.

Authors' Summary

1)*What is Known ?*:

- Neonatal Microbiota at birth resembles that of amniotic fluid, placenta, meconium.

- Microbiome composition changes according to gestational age, mode of delivery, NICU stay, antibiotics, type of feeding, kangaroo mother care etc.

2)What is New ?

- Mycobacterium species can be found of very preterm neonates while their NICU stay in Indian context.

- Kangaroo care could potentially decreased their abundance on the skin.

- Further larger studies are require to know the significance

Introduction

The neonatal skin microbiome consists of all the genomes and genetic products of microorganisms harbouring on the skin of babies[1]. This is mainly constituted by bacteria of phyla *Actinobacteria*, *Proteobacteria, Bacteroidetes, Firmicutes,* and *Tenericutes*[2]. Neonatal microbiota helps in angiogenesis, immune function, intestinal T-cell development, gut-associated lymphoid tissue development, etc. Host and the microbiota develop a harmonious environment resulting in symbiosis. Any disruption of this environment could lead to pathological disease[3]. Preterm neonates during their neonatal unit stay are exposed to various interventions like mechanical ventilation, antibiotics, central lines, tube feeding, kangaroo mother care (KMC) etc which are lifesaving yet come with caveats. KMC is one of the most cost-effective interventions which is proven to reduce mortality and promote early discharge in this cohort[4]. The presence of Mycobacterium species on the skin of preterm neonates is a rarity and has not been reported previously. The clinical significance of their existence on preterm skin also remains elusive.

We incidentally found the presence of Mycobacterium species in very preterm babies (<32 weeks of gestation) admitted to the Neonatal Intensive Care Unit (NICU).

Materials And Methods

The study was conducted to understand the neonatal skin microbiome of very preterm neonates admitted to NICU at a tertiary health care setting before and after KMC. The study included collection of skin swabs of preterm neonates (n-30) before 32 weeks of gestation and admitted to a Level III NICU in a Tertiary Health Care Hospital at Bangalore Medical College and Research Institute (BMC&RI) during June 2020 – December 2020. The skin surface of neonates was swabbed with sterile cotton pledget that had been soaked in sterile normal saline from right axilla, chest, abdomen, groin and to left axilla in a single sweep. The first swab (Sample A) was collected between the 5^{th} -10^{th} day of postnatal life. A second swab (Sample B) was taken after 7 days of adequate KMC (minimum 6 hours per day). The swabs were sent to the genomics laboratory in sterile water for Next-generation sequencing (NGS). Detection was done using credence rapid infection detection (credence RIDTM) Credence Rapid Infection kits for Next Generation Sequencing after Polymerase Chain Reaction (PCR) – amplification. The reads obtained were then compared to standard library reads by matching Barcoded bacterial and fungal libraries that were multiplexed on a single chip on a 400 bp run to obtain sequencing data. Specimens were run in batches of 20 on an Ion 318TMv2 chip (Thermo Fisher Scientific). Data was analysed using Credence Genomics proprietary bioinformatics pipeline for analysis of clinical isolates. Ethical approval was obtained for the study from Institutional Ethics Committee (IEC - BMCRI/PS/184/2020-21).

Results

Data obtained was in the form of relative abundance with respect to the presence of other organisms in the given clinical isolate (swab sample). Percentage of the relative abundances obtained directly from NGS are shown in Table 1. In sample A, the observed average relative abundance for *M.tuberculosis* was identified to be 0.013904093% present in 83.33% (25/30) of samples and for *M.abscessus* 0.116270902% present in100% (30/30) of samples, while in sample B the values observed for *M.tuberculosis* was 0.01142986% in 66.67% (20/30) and for *M.abscessus* was 0.100288231% in 93.33% (28/30) of samples . We found *Mycobacterium tuberculosis* in 83.33% & 66.67% (p-value , 0.285) and *Mycobacteroides abscessus* in 100% & 93.33%(p-value , 0.303) on the skin microbiome before and after KMC respectively. The analysis was done using Wilcoxon signed-rank test (IBM-SPSS v.23, New York, USA) with continuity correction at 95%, and significant p-value taken <0.05.

Table I – Relative abundances(%) of organisms on the skin samples of 30 neonates.

Sample	*Mycobacterium tuberculosis*		*Mycobacteroides abscessus*	
Baby	A(%)	B(%)	A(%)	B(%)
1	0.012534455	0.000268456	0.137414769	0.137717751
2	0.001534876	0.000757722	0.173660218	0.184252766
3	0.039691829	0.001730064	0.005379479	0.080880487
4	0.044313312	0.103367833	0.062038637	0.000108808
5	0.052601143	0.000432548	0.173261271	0.02043789
6	0.001960792	0.001522965	0.223726368	0.172915119
7	0.000739687	0	0.138198172	0.147914607
8	0.000236503	0.001010763	0.029208094	0.062330381
9	0	0	0.109875675	0.203762396
10	0	0	0.201615035	0
11	0	0	0.192187333	0.195936056
12	0.002928456	0	0.123783565	0.038512317
13	0.001336047	0.045632531	0.089960498	0.134814781
14	0.00074651	0.000133041	0.109239306	0.059735329
15	0	0.000135553	0.148654131	0.062354365
16	0.005460584	0.107099194	0.04869949	0.054132563
17	0.000443521	0.048278859	0.090478204	0.045768358
18	0.00195163	0	0.195921961	0.028695038
19	0.023888141	0	0.145371908	0.016083746
20	0	0.000129921	0.142229626	0.101598421
21	0	0.002571256	0.19813293	0.1961654
22	0.03197038	0	0.136306147	0.180660792
23	0.001759527	0.000664643	0.011964781	0.134922494
24	0.001927352	0	0.002248577	0.139318255
25	0.056567622	0	0.034147528	0.170347565
26	0.001232365	0.01829928	0.038066392	0
27	0.004451644	0.001925155	0.165796605	0.120605269

28	0.05738533	0.000938491	0.07886153	0.197396
29	0.071146877	0.001925155	0.079248274	0.120605269
30	0.000314202	0.006072383	0.202450563	0.000674709

Sample A – First swab taken between 5th -10th postnatal day

Sample B – After 7 days of adequate Kangaroo Mother care

Discussion

Neonates, especially preterm babies are susceptible to bacterial or viral infection due to their poor immune response, high sickness level, use of broad-spectrum antibiotics, presence of central lines, delayed establishment of feeding, etc. The development of the neonatal microbiome commences from the womb, where the foetus ingests and is bathed in the hypothetical 'sterile' amniotic fluid[3] . Various studies have proven that neonatal oral microbiota resembles that of amniotic fluid, placenta, meconium[2,5]. Development of skin microbiome begins similarly, and varies in babies born via caesarean section or normal delivery, differences in the postnatal care, duration of intensive care stay, duration and type antibiotic therapy, KMC etc.

KMC, defined as both continuous skin-to-skin contact of the infant with the chest of the mother and exclusive breastfeeding, is among the most effective interventions for preventing death in infants with low birth weight in Low Middle-Income countries[6]. During this process, studies[7] have shown that there is a transfer of healthy microbiota from the mother replacing the pathogenic organisms. Most of the organisms belong to bacterial phyla of *Actinobacteria*, *Proteobacteria, Bacteroidetes, Firmicutes,* and *Tenericutes*[7]. To our knowledge, no study to date has reported the presence of Mycobacterium species in the skin microbiome of preterm neonates whilst their intensive care stay. It was intriguing that we incidentally found these pathogenic species on preterm skin. About 83.33% & 100% of our cohort had *Mycobacterium tuberculosis* & *Mycobacteroides abscessus* respectively in the first swab which could not be explained with certainty. None of the mothers had any history of tuberculosis (any type) in the past. Family history was not available in all cases. After KMC 66.67% & 93.33% of babies had *Mycobacterium tuberculosis* & *Mycobacteroides abscessus* respectively. The relative abundance of these species decreased after KMC, however not statistically significant.

The incidence of congenital tuberculosis is about 2% in high endemic countries which is mainly by mode of vertical transmission[8]. Postnatal transmission from caregivers to babies is also known from parents with active tuberculosis. Infants with congenital tuberculosis may be symptomatic at birth, but symptoms (mainly respiratory) can also occur within days to weeks after birth. Mortality can be as high as 40% due to respiratory failure in most cases[9].

In vulnerable group such as new-born population, as this study confers, is highly susceptible to multiple infectious diseases. The significance of these bacilli as part of cutaneous microbiome, during the NICU

stay, post-discharge, and in the infancy period will only be known on long-term follow-up. None of the babies had any clinical signs of tuberculosis nor any mortality until discharge was attributed to tuberculosis. The mere presence of these bacilli, whether just as commensals or as potential pathogens, is alarming due to the risk of early exposure and incidence of tuberculosis right from birth.

These findings, in our view, are the first findings to be established in such a setting. Mycobacterium species detected incidentally in our study in very high percentages needs exploration with larger cohorts. Although clinically none of the mothers had any history of tuberculosis nor were active cases of tuberculosis, testing all the mothers or any surrounding source would also pose as a limitation of this study. Further larger studies are required to understand the presence of these organisms on preterm skin with long term follow up data including testing both the parents.

Abbreviations

KMC – Kangaroo Mother Care

NGS – Next Generation Sequencing

NICU – Neonatal Intensive Care Unit

Declarations

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Conflicts of interest/Competing interests: Not Applicable

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Code availability: Not Applicable

Authors' contributions: SD: Conceived the idea, designed the study, supervised the data collection, critically appraised the data analysis, drafted the first manuscript, and approved the final manuscript_PS/VG: Data collection, data analysis, reviewed the data, drafted the first manuscript, approved the final manuscript. All authors approved the final manuscript.

Ethics approval: Ethical approval was obtained for the study from Institutional Ethics Committee , Bangalore Medical College and Research Institute. (IEC - BMCRI/PS/184/2020-21).

Consent to participate: Consent Obtained from parents of all neonates enrolled in the study.

Consent for publication: All authors consent for publication upon review and acceptance.

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