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Association Between Rectal Colonization and Risk of Blood Stream Infection with Acinetobacter *Baumannii* in Neonates

Supreet Khurana[1]; Harinder Kaur[3]; Manisha Biswal[3]; Shiv Sajan Saini[2]*; Pallab Ray[3]; Praveen Kumar[2]

[1]Department of Neonatology, Government medical college and hospital, Chandigarh, India.

[2]Division of Neonatology, Department of Pediatrics, Post Graduate Institute of Medical Education and Research, Chandigarh, India.

[3]Department of Microbiology, Post Graduate Institute of Medical Education and Research, Chandigarh, India.

***Corresponding Author(s): Shiv Sajan Saini**

Associate Professor, Division of Neonatology, Department of Pediatrics, Post Graduate Institute of Medical Education and Research, Chandigarh, India.

Tel: +91- 9501112646; Email: sajansaini1@gmail.com

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Abstract

Objective: Gastrointestinal colonization has been proposed as an important risk factor for subsequent Acinetobacter - Blood Stream Infection (ACB-BSI) septicemia, however its association in neonates remains unproven.

Material and methods: In this cohort study, we obtained rectal swabs from all neonates, admitted to Neonatal Intensive Care Unit (NICU) within 48 hours of their stay. Their rectal swabs were repeated at weekly intervals until they either develop ACB-BSI or were shifted out of NICU (whichever was earlier).

Results: A total of 250 rectal swabs were obtained from 155 neonates. Nineteen neonates were colonized with Acinetobacter *baumanii* (24 positive rectal swabs). Out of these 19 neonates, 4 (21%) developed ACB-BSI. Amongst 136 non-colonized neonates, 8 (5.8%) developed ACB-BSI. Gut colonization with Acinetobacter *baumanii* was associated with a significantly higher risk of subsequent ACB-BSI [RR 3.6 (95% CI 1.2- 10.7), p-value 0.02]. Out of 12 isolates of ACB, 11 (4 isolates in cases and 7 in controls) were extremely drug resistant. Three of four neonates, who developed ACB-BSI after gut colonization, died.

Conclusions: Rectal colonization with ACB showed a significant association with subsequent risk of Acinetobacter *baumanii* blood stream infection in neonates admitted in NICU. A very high incidence of extremely drug-resistant Acinetobacter *baumanii* was reported.

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Introduction

Acinetobacter *Baumannii* has been labelled by World Health Organization as leading critical pathogen emerging as multidrug resistant and extensively drug resistant strain globally [1]. Acinetobacter, (ACB) a gram negative strictly aerobic, non-fastidious coccobacillus, is currently a common causative organism of neonatal sepsis [2]. Delhi Neonatal Infection Study (DeNIS) study observed Acinetobacter spp. as the most common causative organism responsible for 22% of all episodes of neonatal sepsis [3]. Additionally, ACB had a high rate of multi-drug resistance and was associated with a case fatality rate of 59% [3]. The outcomes of the patients infected with Multi-Drug Resistant (MDR) ACB are worse than those who were not infected with MDR-ACB [4]. Many risk factors for Acinetobacter septicemia are identified in neonates such as institutional delivery, prematurity, very low birth weight, prolonged hospitalization of >7 days, catheterization, endotracheal intubation, parenteral nutrition, broad spectrum antibiotic therapy, and artificial ventilation, immune-compromised status, inadequate hand hygiene, and surface contamination [5,6].

One of the proposed mechanisms of Acinetobacter infection is through colonization of gastrointestinal (GI) tract [7]. The gut of the neonates gets colonized with microbiome acquired through perinatal transfer from maternal genital tract, breast milk, periodontal flora of mother, from contaminated environmental surfaces, procedures and human contact (hands, nasal carriage etc.) [8]. Sick neonates admitted in NICU or neonatal nurseries are at higher risk of getting colonized with resistant organisms from hospital environment [7-9]. The colonization of the GI tract has been linked to increased risk of Blood Stream Infection (BSI) with pathogenic bacteria especially gram-negative bacteria [7]. Literature regarding asymptomatic colonization of neonates admitted in NICU is available, [8] however studies identifying the association between gastrointestinal colonization of the neonates by ACB and development of subsequent Blood Stream Infection (BSI) are lacking. It is important to identify causal mechanisms that lead to invasive infection and dissemination of this pathogenic bacteria. We conducted this study to identify the association between rectal colonization of ACB and risk of acquiring subsequent ACB-BSI among neonates admitted in a level-III NICU.

Material and methods

This prospective cohort study was carried out in a level-III NICU of a tertiary care referral institution from August 2015 to January 2016. The study was approved by Institutional Ethics Committee.

Participants

All intramural neonates admitted in NICU with a minimum stay of 48 hours were eligible to be included in the study. We excluded neonates if they had lethal congenital malformations, primary or secondary immunodeficiencies, anorectal malformations, admitted in a moribund state, hemodynamic instability, were shifted out of NICU within 48 hours of admission, and already diagnosed with Acinetobacter *baumannii* sepsis. The neonates were enrolled after obtaining a written and informed consent from one of the parents.

Protocol: All enrolled neonates were attached to the multichannel monitor and kept under continuous observation. Their baseline data-demographic details, maternal risk factors (namely leaking per vaginum ≥18 hours, clinical chorioamnionitis, maternal urinary tract infection, maternal fever, maternal loose stools), use of Intravenous (IV) antibiotics, mechanical ventilation (non-invasive/invasive)-were recorded. After 48 hours of NICU stay, rectal swabs were obtained from all the enrolled neonates. The rectal swabs were collected every week on fixed days (Tuesday between 9-10 AM), aseptically by the resident doctors. The neonates were turned to the left lateral decubitus position. Sterile cotton swab sticks were inserted up to 2-3 cm in the anal canal in neonates weighing <2000 gm birth weight and up to 4-5 cm in neonates weighing >2000 gm. After collection, the swab sticks were placed back into the sterile glass tubes and sealed with the help of air-tight tube lids. The rectal swabs were transported to the department of Medical Microbiology within 1-hour of collection. In all enrolled neonates who continued to stay in NICU for more than 7 days, the rectal swabs were repeated at weekly intervals irrespective of previous swab status till the neonates were shifted out of NICU or developed Acinetobacter blood stream infection. The post-natal age at time of rectal colonization with Acinetobacter *baumannii* and age of rectal clearance was recorded.

All enrolled neonates were followed for the development of blood stream infections during their stay in NICU. The neonates developing clinical signs suggestive of neonatal sepsis were investigated using septic workup including blood culture. We defined culture proven sepsis/ Blood Stream Infection (BSI) by isolation of microorganism from the sterile body fluid. We defined probable sepsis using nosocomial infection episode description criteria adapted from NEOKISS protocol for Nosocomial infection surveillance for neonates [10]. We performed CSF analysis in all symptomatic neonates to rule out meningitis. All neonates were followed up till mortality or discharge from hospital, whichever came earlier.

Laboratory procedure: Within two hours of collection, the rectal swabs were inoculated into MacConkey agar. Identification of suspected colonies was done by Matrix Assisted Laser Desorption/ Ionization- Time of Flight Mass Spectrometry (MALDI-TOF) (Bruker Daltonik, Bremen, Germany). Spectra were acquired and recorded in the positive linear mode at a laser frequency of 20 Hz, ion source 1 voltage of 20 kV, ion source 2 voltages of 8.5 kV, and mass range from 2000 to 20,000 Da. A score of >1.8 was taken as diagnostic of Acinetobacter *baumannii*. The antibiotic susceptibility of ACB was performed using Kirby Bauer disc diffusion test following CLSI guidelines [11]. Blood cultures, obtained as a part of septic work-up, were processed in automated blood culture system (BACTEC, Becton Dickinson, USA). Blood culture were taken as positive if bacterial growth was identified within first 48 hours of inoculation.

Outcome measures

Our primary outcome measures were blood culture proven ACB sepsis in the NICU. All neonates with culture-proven ACB septicemia were categorized as MBSI-ACB group (microbiologically confirmed blood stream infection with Acinetobacter *baumannii*) and without ACB sepsis were categorized as either Clinical Sepsis (C-SEP) or MBSI (Microbiologically confirmed Blood Stream Infection with non-CONS) or MBSI-CONS (Microbiologically confirmed Blood Stream Infection with Coagulase Negative Staphylococci) as sole pathogen or Meningitis (MEN) depending upon clinical presentation and investigations. Our secondary outcome measures were mortality, clinical sepsis, meningitis and blood stream infection with other micro-organisms.

Statistical analysis

Descriptive statistics were used to define the baseline demographic and clinical characteristics. Baseline characteristics were compared between cases and controls using students' t-test or Mann-Whitney 'U' test for continuous variables and chi square test with Yate's correction or Fischer exact test for proportions between. We included only the first rectal isolate in the calculation for data analysis. We estimated relative risk along-with 95% confidence interval of ACB-MSI among ACB gut-colonized neonates (cases) and non-colonized neonates (controls). Analysis was performed using SPSS version 26.0 (IBM, New York).

Results

We assessed 239 neonates admitted to NICU for eligibility during the study period. 84 neonates were excluded (51 were stabilized and shifted to step down nursery before rectal sampling, 2 were extramural neonates, 2 had critical congenital heart disease requiring surgical intervention, 1 had ano-rectal malformation, 9 were moribund at time of admission while 16 died before sampling and parents of 3 neonates left treatment against medical advice). We enrolled 155 neonates and obtained 250 rectal swabs from them. Out of 155 neonates, rectal swabs of 19 (12.2%) neonates were found to be colonized with ACB with a swab positivity rate of 9.6% (24 out of 250) "Figure 1". 2 out of 19 neonates were negative on initial swab and turned positive on subsequent swabbing. The baseline characteristics of neonates with positive rectal swabs (cases) and non-colonized neonates (controls) are presented in "Table 1". Higher proportion of cases required ventilation (non-invasive and/or invasive) as compared to controls. During the study period, we recorded 29 episodes of microbiologically confirmed blood-stream infections. Of them 12 (41%) isolated were *Acinetobacter baumanii*, *E. coli* (n=6, 27%), *Klebsiella pneumoniae* (n=5, 23%), and 2-each were of *Streptococcus mitis*, *Staphylococcus aureus* & *Staphylococcus hemolyticus.*

Out of 19 cases (gut colonized with ACB), four (25%) developed ACB-BSI within 1.5 (±1) days of follow-up. In contrast, 8 neonates amongst 136 controls (non-colonized neonates) developed ACB-BSI (5.8%). Gut colonization was found to be significantly associated with subsequent risk of ACB-BSI [RR 3.6 [95% confidence interval 1.2- 10.7, p value-0.02]. Cases and controls had comparable risk of blood stream infection with microorganisms other than *Acinetobacter baumannii*, clinical sepsis, meningitis and mortality "Table 2". *Acinetobacter baumanii* isolated from all 4 cases and 7 out of 8 controls with both rectal as well as blood-stream ACB positivity were extremely drug resistant as it was susceptible to only colistin. The characteristics of our four cases having *Acinetobacter* gut colonization and blood stream infection are described in "Table 3".

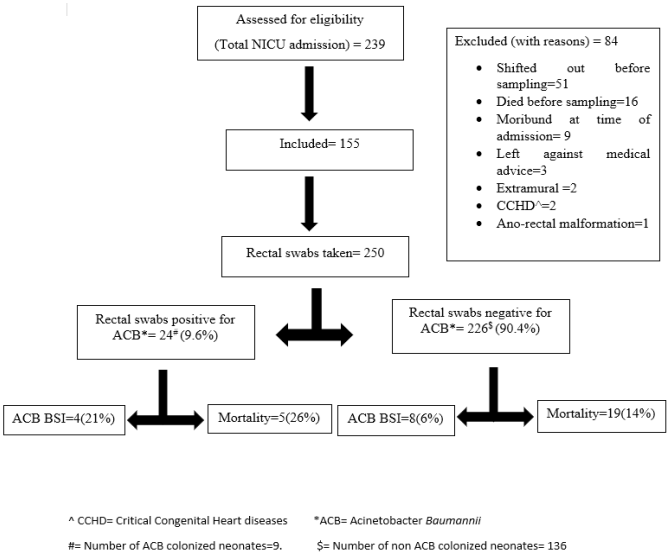

Figure 1: Figure depicting flow of patients during study period.

Table 1: Baseline characteristics of neonates.

Characteristics	ACB* Swab positive neonates# (n=19)	ACB* Swab Negative Neonates# (n=136)
Gestational age (weeks)	31.7 ± 3.3	31.4 ± 3.4
Birth weight (grams)	1172 ± 728	1250 ± 633
Postnatal age (days)	8 ± 4.6	7 ± 7.6
Maternal risk factors of infection (%)	5 (26)	37 (27)
Male Gender (%)	9 (47)	83 (61)
Receiving antibiotics (%)	18 (95)	121 (89)
Receiving Invasive or Non-invasive Ventilation (%)	19 (100)	107 (78)

*ACB=Acinetobacter *Baumannii,* # The values in the table are either mean (± SD) or numbers (percentages) as applicable.

Table 2: Outcome variables of Acinetobacter *Baumannii* colonized and non-colonized neonates.

Variable	ACB* Swab positive neonates# (n=19)	ACB* Swab negative neonates# (n=136)	RR (95% CI)	p- value
Acinetobacter blood stream infection (%)	4 (21)	8 (6)	3.6 (1.2 - 10.7)	0.02
BSI ^ other than Acinetobacter (%)	4 (21)	18 (13.2)	1.6 (0.6 - 4.2)	0.48
Clinical Sepsis (%)	6 (32)	44 (32)	1.0 (0.5 -1.9)	0.94
Meningitis (%)	3 (16)	15 (11)	1.4 (0.4 - 4.5)	0.46
Mortality (%)	5 (26)	19 (14)	1.9 (0.8-4.4)	0.18

*ACB=Acinetobacter *Baumannii,* # The values in the table are either mean (± SD) or numbers (percentages) as applicable.

Table 3: Characteristics of cases which developed Acinetobacter blood stream infection.

Gestation(weeks)	34	29	32	28
Birth weight (grams)	1500	1250	1172	848
Gender	Female	Male	Female	Female
Maternal risk factors	No	Yes	Yes	No
Age at sending rectal swab (day of life)	11	9	14	6
Receiving antibiotics	Yes	Yes	Yes	Yes
Receiving Ventilation (Invasive/Noninvasive)	Yes	Yes	Yes	Yes
Acinetobacter Blood Stream Infection	Yes	Yes	Yes	Yes
Other Blood Stream Infection	No	No	No	No
Clinical Sepsis	No	No	No	No
Meningitis	No	Yes	No	No
Rectal swab strain antibiotic susceptibility	Resistant to all except Colistin	Resistant to all except ampicillin-sulbactam, Colistin, tetracycline	Resistant to all except Colistin	Resistant to all except Colistin
Blood culture strain antibiotic susceptibility	Resistant to all except Colistin	Resistant to all except Colistin	Resistant to all except Colistin	Resistant to all except Colistin
Outcome	Died	Died	Recovered	Died

Discussion

In our cohort study, we observed a rectal colonization rate of 12% among neonates admitted to NICU. We found a positive association between the rectal colonization with ACB and subsequent risk of ACB blood-stream infections among neonates admitted in NICU.

After birth nascent neonatal gut gets rapidly colonized with microflora, which is acquired either from maternal vaginal tract or environmental. Sick neonates, particularly premature neonates who undergo procedures, tend to get colonized with pathogenic and multidrug resistant bacteria from the hospital environment [12]. Bacterial overgrowth in neonatal gastrointestinal tract has been linked to neonatal sepsis [13]. The microorganisms from neonatal gut could directly translocate to the blood through immature or diseased bowel wall. Alternatively, immature defense mechanisms of neonates (especially premature neonates) may aid in indirect transfer via other pathways [13]. Epidemiological data of hospital outbreaks also shows relationship between the colonizing microorganisms and BSI among septic neonates [13,14].

Our data is in agreement with previously published studies about gut colonization by gram negative bacilli and the risk of BSI. Almuneef et al observed that 56% of neonates in NICU were colonized with gram-negative rods (8). They recorded 14 episodes of blood stream infections [E. coli (n=10) and K. pneumoniae (n=4)] in these neonates. They typed 12 of these 14 episodes using molecular methods and observed that 10 neonates were infected with the previously colonized strain. Singh and colleagues found 17% incidence of health care-associated antimicrobial non-susceptible *Enterobacteriaceae* colonization among 1,410 neonates over 3 years, of which 34 (14%) developed infections with *Enterobacteriaceae* [15]. Parm and colleagues evaluated role of surveillance cultures as a risk of late-onset sepsis in neonates. They found a significant association between getting colonized with *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *E. coli*, *Stenotrophomonas spp*. and *Pseudomonas spp*. and late-onset sepsis. However, in their study colonization with *Acinetobacter* was not associated with risk of Late Onset Sepsis (LOS). They had only two colonized neonates prior to *Acinetobacter* LOS, which is the most likely reason for difference in their findings [16]. Graham and colleagues observed 95% genotypic concordance in 95% of bloodstream infections and antecedent rectal cultures among neonates <1500gm admitted in NICU [17]. Majority of these studies have been done in high-income countries. From Low- Middle Income Countries (LMIC) countries, Das and colleagues showed that the neonates having gut colonization with gram-negative bacilli had consistently higher incidence of clinical sepsis as compared to non-colonized neonates [14]. The genotyping data revealed 75% similarity between GNB in blood and gut. In the current study, we observed a significant increased risk of *Acinetobacter*-BSI among neonates with rectal-colonization with *Acinetobacter* in comparison to non-colonized neonates.

We observed a very high incidence of extremely drug resistance of ACB in our study. Furthermore, cases with subsequent ACB-BSI had a very high cases fatality rate (3or 4 died). This data is in accordance with the data recently published in DeNIS study. Therefore, analysis of colonization pattern may be helpful to inform clinical practice. We observed an increased risk of subsequent BSI after colonization. Therefore, a closer monitoring of ACB-colonized neonates may make a difference in their outcome. Additionally, the information of colonization patterns can be helpful to adjust choice of empirical antibiotic regimens. Furthermore, in the setting of intensive care colonization type can be used to plan strategies for prevention of cross-infections and to carve strategies, such as patient cohorting. Future studies can also look at the impact of such strategies on the prevention of BSI in neonatal nurseries. Such data along with microbial susceptibility can also be utilized as an active surveillance to measure development of resistant strains as well as a red flag for probable epidemics in NICUs.

Our study had some limitations. The event rate i.e., microbiologically confirmed BSI was low, which resulted in the wide

confidence intervals in our primary outcome. Therefore, the certainty of outcomes would have been better, had we recruited more subjects. We did not perform molecular methods for genotyping of the colonized and BSI ACB. Hence, we are not sure of the true concordance between colonized and BSI *Acinetobacter*. We did not check for colonization at any other site. apart from rectum. The other possible places of colonization -like skin, intravenous cannula sites could have also contributed to the BSI's. We did not check for the other GNB colonization, which could be helpful to establish the risk of subsequent BSI after colonization with these organisms. The future studies should be designed to overcome these limitations.

Conclusion

In this prospective cohort study, we observed 12% *Acinetobacter* rectal colonization rates among 155 neonates after initial 48 hours of NICU stay. We found a statistically significant association between the rectal colonization of neonates with *Acinetobacter* and subsequent risk of microbiologically confirmed blood stream infection with *Acinetobacter baumanii*.

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