Reservoirs of transmission of resistant Gram-negative pathogens responsible for neonatal sepsis among hospitalized neonates in Pune, India

Julia Johnson[1], Matthew L. Robinson[2], Shilpa Naik[3], Sunil Patil[3], Rajesh Kulkarni[4], Aarti Kinikar[4], Vaishali Dohe[5], Swati Mudshinkar[5], Anju Kagal[5], Rachel M. Smith[6], Matthew Westercamp[6], Bharat Randive[7], Abhay Kadam[7], Vandana Kulkarni[7], Vidya Mave[2,7], Amita Gupta[2], Aaron M. Milstone[8], Yukari C. Manabe[2], for the CDC SHEPheRD India Study Team

[1]Division of Neonatology, Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA, [2]Division of Infectious Diseases, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA, [3]Department of Obstetrics, Byramjee Jeejeebhoy Government Medical College, Pune, India, [4]Department of Pediatrics, Byramjee Jeejeebhoy Government Medical College, Pune, India, [5]Department of Microbiology, Byramjee Jeejeebhoy Government Medical College, Pune, India, [6]Centers for Disease Control and Prevention, Atlanta, GA, USA, [7]Byramjee Jeejeebhoy Government Medical College-Johns Hopkins University Clinical Research Site, Pune, India, [8]Division of Pediatric Infectious Diseases, Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Julia Johnson, MD
1800 Orleans Street
Suite 8534
Baltimore, MD 21287
jjohn245@jhmi.edu

Abstract

Background: Neonatal infections with resistant Gram-negative (GN) organisms are associated with high rates of mortality, with limited antibiotic treatment options. The role of maternal colonization and environmental GN organisms as reservoirs for transmission to neonates has not been well described.

Methods: We performed a prospective cohort study from October 12, 2018, until October 31, 2019, to describe the role of maternal and environmental GN colonization in BSI among neonates admitted to the neonatal intensive care unit (NICU) at Byramjee Jeejeebhoy Government Medical College in Pune, India. Women admitted to Labor & Delivery with risk factors for neonatal sepsis who provided consent were enrolled and their neonates were followed until hospital discharge. For neonates who developed bloodstream infection (BSI), colonization with resistant GN organisms was assessed in their mothers from frozen vaginal and rectal swabs collected at enrollment and at delivery and in the neonates from frozen skin swabs and peri-rectal swabs collected at day of life (DOL) 0, 3, 7, and weekly until discharge. Environmental colonization was assessed with weekly sampling of unit sinks and the immediate neonatal care environment. Colonization samples were processed to identify organisms that matched neonatal blood culture isolates.

Results: 953 women were enrolled, of whom 741 (78%) received antepartum antibiotics. Among 987 live born neonates, 12 (1%) died in the delivery room and 257 (26%) required NICU admission. Among neonates admitted to the NICU, 143 (56%) had at least one blood culture, of which 28 (20%) were positive; 21 (75%) had a GN BSI. The most common cause of neonatal BSI was *Klebsiella pneumoniae*, and 8 (38%) GN BSI were due to a carbapenem-resistant organism. Matching strains were found in maternal rectal samples, neonatal peri-rectal and skin samples, and unit sinks.

Conclusion: Among neonates born to mothers with risk factors for neonatal sepsis, GN organisms were the most common cause of neonatal BSI. Environmental and neonatal colonization may represent important reservoirs of transmission for these pathogens among neonates hospitalized in a tertiary care NICU in Pune, India.

Background

- Facility-based births are increasing in low and middle income countries (LMICs), and healthcare facilities are increasingly caring for preterm and sick neonates.
- BSIs among hospitalized neonates in LMICs, including India, are commonly caused by GN pathogens, with high rates of antimicrobial resistance (AMR).
- Reservoirs of transmission are poorly described, limiting capacity to prevent these infections.

Methods

- We conducted a prospective cohort study from October 12, 2018, until October 31, 2019, to describe the role of maternal and environmental GN colonization in BSI among neonates admitted to the NICU at a tertiary care center in Pune, India.
- Women admitted to Labor & Delivery with risk factors for neonatal sepsis who provided consent were enrolled and their neonates were followed until discharge.
- Neonatal blood cultures were obtained at discretion of the clinical team at time of suspected sepsis and were processed in the microbiology laboratory per clinical practice.
- Colonization with resistant GN organisms was assessed in mothers from vaginal and rectal swabs collected at enrollment and at delivery and in neonates from skin swabs and peri-rectal swabs collected at day of life DOL 0, 3, 7, and weekly until discharge.
- Environmental colonization was assessed with weekly sampling of unit sinks and the immediate neonatal care environment.
- All colonization samples were collected using the Eswab collection system (COPAN FLOQSwabs, 1 ml Liquid Amies medium) and frozen at -80˚ Celsius. For neonates who had a positive blood culture, maternal, neonatal, and environmental samples obtained prior to the positive blood culture were thawed. Broth enrichment was followed by plating an aliquot on agar plates. Isolates were analyzed using VITEK for identification and antimicrobial susceptibility testing.
- This study was approved by the Johns Hopkins Medicine Institutional Review Board and the Byramjee Jeejeebhoy Government Medical College Ethics Committee.

Results

Table 1. Baseline clinical and demographic characteristics of pregnant women

	Total (n=953)
Maternal age in years, median (IQR)	23 (21-26)
Gestational age at admission in weeks, median (IQR)	36 (34-38)
Multiple gestation, n (%)	50 (5)
Pre-gestational diabetes, n (%)	7 (1)
Gestational diabetes requiring insulin therapy, n (%)	16 (2)
Preeclampsia, n (%)	10 (1)
Antenatal steroids within 14 days of admission, n (%)	511 (54)
Premature rupture of membranes (PROM), n (%)	363 (38)
Duration of rupture of membranes in hours, median (IQR)	13 (6-23)
Meconium-stained fluids, n (%)	289 (30)
Maternal antepartum fever, n (%)	2 (0)
Antepartum antibiotics, n (%)	741 (78)
Number of vaginal exams prior to delivery, median (IQR)	6 (4-8)

Figure 1. Study flow diagram

Table 2. Baseline characteristics of neonates admitted to the NICU

	Total (n=257)
Male, n (%)	142 (55)
Gestational age in weeks, mean (SD)	34 (3)
Birth weight in grams, mean (SD)	1773 (569)
Low birth weight, n (%)	195 (89)
Multiple gestation, n (%)	48 (19)
Cesarean delivery, n (%)	63 (29)
Positive pressure ventilation at birth, n (%)	43 (20)
Mechanical ventilation on admission, n (%)	312 (9)
Central line on admission, n (%)	5 (2)
Pressors on admission, n (%)	10 (5)
Antibiotics on admission, n (%)	130 ()

- Among 28 neonates with a positive blood culture, 21 (75%) had a BSI with a GN pathogen, of which 15 (71%) were resistant to 3rd or 4th generation cephalosporins and 8 (38%) were resistant to carbapenems.
- Age at time of positive blood culture ranged from 0 to 30 days of life; 5 (24%) were early onset BSI (DOL 0-2) and 16 (76%) were late onset BSI (DOL 3 or later).

Table 3. Neonatal Gram-negative bloodstream infections by bacterial genus, age at onset, resistance, and matching organism from maternal, neonatal, and environmental samples

Bacterial genus	Age at BSI in days, range	Resistance by antibiotic class, n (%)	Matching organism by source and resistance pattern, n (%)
Klebsiella pneumoniae (n=8)	0-17	3rd/4th gen. cephalosporin, 8 (100) Carbapenem, 6 (75)	Maternal rectal, different resistance, 2 (25) Neonatal peri-rectal, same resistance, 3 (38) Neonatal skin, same resistance, 3 (38) Unit sink, same resistance, 3 (38) Unit sink, different resistance, 3 (38)
Pseudomonas spp. (n=4), including 3 identified as *P. aeruginosa*	0-10	3rd/4th gen. cephalosporin, 2 (50) Carbapenem, 1 (25)	No matching organisms isolated from any source
Burkholderia cepacia (n=4)	0-30	3rd/4th gen. cephalosporin, 2 (50) Carbapenem, 0	No matching organisms isolated from any source
Citrobacter spp. (n=2)	8-11	3rd/4th gen. cephalosporin, 1 (50) Carbapenem, 0	No matching organisms isolated from any source
Acinetobacter spp. (n=2) including *A. baumannii* (n=1), *A. lwoffii* (n=1)	1-9	3rd/4th gen. cephalosporin, 2 (100) Carbapenem, 1 (50)	Unit sink, different resistance, 1 (50)
Enterobacter spp. (n=1)	11	3rd/4th gen. cephalosporin, Carbapenem, 0	No matching organisms isolated from any source

Results

Figure 2. Maternal, neonatal, and environmental colonization

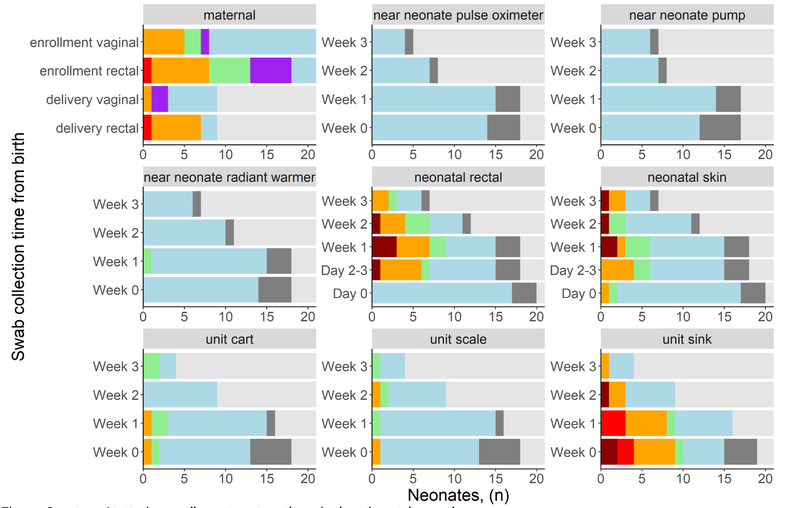

Figure 2 notes. At study enrollment, maternal vaginal and rectal samples were collected. If delivery occurred >6 hours after enrollment, maternal vaginal and rectal samples were repeated at delivery. Neonatal composite skin and peri-rectal samples were collected on DOL 0, 3, 7, and weekly until discharge. Environmental samples included weekly samples of unit sinks and scales and the near-neonate environment (bed, pulse oximeter, pump).

- Among 21 neonates with GN BSI, there were matching organisms identified from maternal rectal swabs (n=2, 10%), neonatal peri-rectal swabs (n=3, 14%), neonatal skin swabs (n=3, 14%), and unit sinks (n=7, 33%).
- No matching organisms were identified from maternal vaginal samples.
- There was limited growth from environmental sources other than the unit sink, with no matching organisms identified.
- Among GN BSI pathogens, the greatest recovery of matching organisms from maternal, neonatal, and environmental sources was for *K. pneumoniae*; there were no matching organisms identified for *Pseudomonas* spp., *Burkholderia* spp., *Citrobacter* spp., and *Enterobacter* spp. and only 1 matching isolate from a unit sink for *Acinetobacter* spp.
- Among the 257 neonates requiring NICU admission, 23 (9%) neonates died and the median length of stay was 7 days (IQR 3-15).
- Among 21 neonates with GN BSI, 4 (19%) died and median length of stay was 17 days (IQR 10-30).

Conclusions

- GN pathogens were the most common cause of BSI among neonates admitted to the NICU in this cohort study; AMR was prevalent. Mortality in neonates with GN BSI was higher than in the full cohort.
- Among neonates with GN BSI, preceding neonatal skin and rectal colonization with matching organisms was identified in a small proportion of neonates with *K. pneumoniae* BSI.
- Maternal colonization does not appear to be a clear source of transmission for neonatal GN BSI in this population.
- Next steps include next-generation sequencing to evaluate strain relatedness between GN blood culture isolates and organisms identified from maternal, neonatal, and environmental colonization samples.

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