Burkholderia cepacia complex - An Outbreak in Neonatal Intensive Care Unit from a Tertiary Care Hospital of Central India

Running title: Burkholderia cepacia complex outbreak in neonates

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Abstract

Background: *Burkholderia cepacia* complex is our opportunistic nosocomial pathogen that can cause severe infections in neonates, involving the respiratory tract, the urinary tract and bloodstream infections. Therefore, it can lead to outbreaks through different sources. This study was conducted with the aim of early detection and successful control of an outbreak caused by *Burkholderia cepacia* complex.

Methods: A cross-sectional study was conducted in a tertiary care hospital over a one-month period, July 2023. Blood culture samples of 11 neonate's yielded growth of non-fermenting, oxidase-positive and motile, Gram-negative bacilli. Isolates were provisionally identified to be *Burkholderia cepacia* complex by conventional biochemical tests and antimicrobial susceptibility patterns. The increased, repeated, and continuous isolation of the same isolate raised the suspicion of an outbreak in the neonatal intensive care unit. Active surveillance was undertaken to trace the source and contain the bacteria. Identification of isolates was confirmed by VITEK 2 (BioMérieux, France) compact microbiology analyser.

Results: Surveillance revealed sources of *Burkholderia cepacia* complex for all 11 neonates. Sources of infection could be traced to intravenous catheters and cradles of the neonates and operation theatre beds, and instrument trolleys of the labour room where the babies were delivered. All the environmental isolates showed strain-relatedness of *Burkholderia cepacia* complex with the clinical isolates, along with a similar antibiotic susceptibility pattern. Timely interventions aided in the control of the outbreak.

Conclusion: This study presents the importance of the hospital infection control team in the management of an outbreak of *Burkholderia cepacia* complex in neonates.

Keywords: *Burkholderia cepacia* complex; Disease outbreaks; Intensive Care Unit; Nosocomial; Sepsis

Introduction

The *Burkholderia cepacia* complex (BCC) initially emerged in the 1980s as an opportunistic human pathogen causing severe and life-threatening infections. It is a group of Gram-negative nonfermenting aerobic betaproteobacteria, associated with various virulence mechanisms such as multidrug resistance (bcrA efflux pump), genes determining transmissibility (esmR and cblA genes, and esmR), siderophores (salicylic acid, ornibactin, pyochelin, and cepabactin), and adherence proteins (long flexible type II pili) (1,2). They are known to withstand disinfection and sterilization procedures as they display a moderate to high-tolerance to stressors such as UV-C radiation, antibiotics, and high heavy-metal concentrations. Hence an appropriate antimicrobial therapy is challenging. The United States Food and Drug Administration (FDA) has proposed the inclusion of these bacteria in the "Objectionable Microorganisms" category (3). Sick neonates admitted to neonatal units in low-resource settings are at an increased risk of developing nosocomial infections due to poor clinical care practices. Neonatal sepsis is defined as the presence of systemic features (fever, tachycardia, tachypnoea, and hypotension) associated with pure growth of bacteria from one or more sites (4).

This article describes the investigation of a BCC outbreak in neonates within the Neonatal Intensive Care Unit (NICU) over the course of one month. The active involvement of the hospital's infection control team was pivotal in successfully identifying the source of the outbreak. Timely and targeted interventions, based on these findings, were essential in containing and controlling the spread of the infection.

Methods

A cross-sectional study was conducted in a tertiary care hospital over one month, July 2023. A total of 11 neonates, clinically suspected of sepsis, were referred by clinicians from the NICU for blood culture investigation in July 2023. Biological parameters like elevated CRP levels (≥6 mg/dl), leucocytosis, and thrombocytopenia were noted. Blood cultures were received in the Department of Microbiology, Government Medical College, Nagpur as a part of routine diagnostic services. Conventional blood culture bottles (5) containing brain–heart infusion broth with 0.025% sodium polyanethol sulphonate were used. Blood culture samples were sub-cultured on blood agar, chocolate agar and MacConkey agar as per the standard protocol (6). Typical smooth, grey, translucent colonies were observed on blood agar & non-lactose fermenting (NLF) colonies on MacConkey's agar (Figure 1). Gram-negative, motile, catalase and oxidase-positive isolates were further identified to the species level by conventional biochemical tests (6,7) as shown in Figure 2. The isolates were confirmed by the automated VITEK 2 (BioMérieux, France) compact microbiology analyser. Antimicrobial susceptibility was determined by both the modified Kirby-Bauer's disk diffusion method and minimum inhibitory concentration (MIC) by the VITEK 2 antimicrobial susceptibility testing (AST) card in accordance with the Clinical and Laboratory Standards Institute (2023) recommendations (8). All the media, biochemicals and disks for antimicrobial susceptibility testing were procured from HiMedia, India.

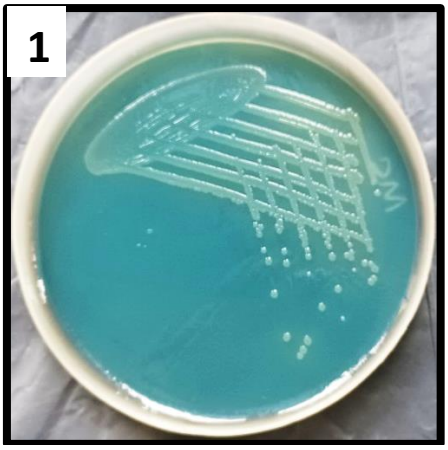

Figure 1. NLF translucent moist colonies on MacConkey's agar

Figure 2. Biochemicals- TSI: K/K, no gas and H_2S, Citrate utilised, Urea not hydrolysed, Indole negative, Methyl Red negative, Base without any added amino acid showing viability of the organism, Ornithine not decarboxylated, Lysine decarboxylated, Arginine hydrolysed

All the isolates from the blood culture samples of the 11 NICU patients revealed oxidase positive, motile, non-fermenting gram-negative bacilli (NFGNB) which were lysine and arginine positive, ornithine negative, resistant to polymyxin B 300U and aminoglycosides. The increased, repeated and continued isolation of oxidase positive NFGNB raised the suspicion of a possible outbreak confined to NICU patients. Presumptive reports were immediately conveyed to the concerned intensivists and an active surveillance was initiated and continued over the month of July 2023.

Environmental surveillance was conducted to trace the source and route of infection. Samples collected were tap water, IV fluids (fresh and opened), distilled water for humidifiers, water for feeds and antiseptic solutions. Pre-moistened sterile swabs were used to collect samples from surfaces like medicine trolley, Ambu bag, cradles, etc (Table 1).

Table 1. Swabs collected for environmental surveillance

Hospital ward - NICU	
Swabs	**Result**
Cradle	BCC
Ambu bag	No growth
Intravenous (IV) catheter hub	BCC
Nursing station	Kpn
Medicine trolley	Kpn
Weighing machine	CoNS
Phototherapy unit	No growth
Hands of healthcare staff	Kpn
Normal saline and antibiotic vials	No growth
Tap water	GPB
Hospital ward - Labour room	
Operation theatre table	BCC
Instrument trolley	BCC
Weighing machine	CoNS
Cradle	CoNS
Surfaces	*E. coli*
Hands of healthcare staff	CoNS

Abbreviations: CoNS - Coagulase Negative *Staphylococcus,* Kpn- *Klebsiella pneumoniae*, BCC- *Burkholderia cepacia* complex*,* GPB- Gram-positive bacilli

The samples were inoculated on blood agar and MacConkey agar plates which were incubated for 48 h at 37°C (9). Tap water samples were centrifuged at 3000 rpm for 15 min and the sediment was processed. All liquid samples were inoculated directly on blood agar, MacConkey agar and in brain–heart infusion broth (1:5 dilution). Plates were incubated at 37°C for 2 days. Brain-heart infusion was incubated at 37°C for 5 days and checked for turbidity daily (10). Subculture from brain–heart infusion was made on blood agar and MacConkey agar plates on the 5th day or earlier in case of turbidity. Environmental isolates were confirmed by automated VITEK 2 (BioMérieux, France) compact microbiology analyser methods.

For our study, an outbreak was defined as simultaneous presence of more than two patients with positive culture results for BCC. Outbreak cases were defined as neonates with a clinical suspicion of sepsis (fever, tachycardia, tachypnoea, leukocytosis or leukopenia, with or without hypotension) who had one or more BCC-positive blood cultures.

Results

All 11 clinically suspected cases of neonatal sepsis were positive for BCC bacteraemia. Table 2 showed that the major neonatal risk factor associated with the infection was preterm birth (73 %) followed by LBW (36%). The minimum age of birth noted was 28 weeks, and the lowest birth weight was 1.6 kg.

Table 2. Risk factors associated with *Burkholderia cepacia* complex infection (n=11)

Neonatal risk factors	Number of neonates involved (%)
Preterm	8 (73)
Low birth weight	4 (36)
Hyperbilirubinemia	3 (27)
Respiratory distress syndrome	1 (09)
Multiple organ dysfunction	1 (09)
Congenital anomaly	1 (09)

The environmental surveillance revealed the sources of BCC to be IV catheters and cradles of the patients in NICU, OT tables and instrument trolleys of the labour room as already shown in Table 1.

Biochemical tests and antimicrobial susceptibility pattern showed strain relatedness to that of the isolates recovered from blood cultures of the neonates. Antimicrobial susceptibility testing revealed that all strains were susceptible to levofloxacin, cotrimoxazole and minocycline, with few being resistant to meropenem and ceftazidime.

Discussion

In the present study, all the 11 BCC blood culture positive neonates were clinically diagnosed as cases of sepsis with varying antecedent conditions such as preterm birth, LBW, RDS, hyperbilirubinemia and congenital anomaly (tetralogy of Fallot). The most commonly associated neonatal risk factors were found to be preterm birth (gestational age < 37 weeks), followed by low birth weight (< 2.5 kg) and hyperbilirubinemia. Our findings were similar to the study by Belachew et al (11) and Murthy et al (12).

A lot of medical professionals give antibiotics without first culturing the infection's focal site. Hence, arises the threat of nosocomial dissemination of multidrug resistant organisms like BCC. Various studies have shown higher susceptibility for meropenem and ceftazidime (13,14). However, it was quite low in our study. We found highest susceptibility to levofloxacin followed by co-trimoxazole and minocycline. Considering the resistance profile of the isolates, levofloxacin was deemed the most effective treatment option. With careful medical supervision, the potential benefits of levofloxacin were considered to outweigh the risks, leading to the initiation of therapy.

Despite of treatment, persistent sepsis with no clinical improvement were noted in all the neonates. Based on antimicrobial susceptibility testing report, the antibiotic therapy was modified. 7 of the patients responded well to the treatment while 4 neonates succumbed. Two of them died as a result of disseminated intravascular coagulation (DIC) and antecedent causes being LBW and RDS. The cause of death of the third baby was sepsis shock. This baby had acyanotic congenital heart disease. The fourth baby died of sepsis with DIC and antecedent causes being hypoxia induced encephalopathy (HIE), preterm and LBW.

Surveillance findings suggested that BCC isolated from the surface of the OT beds and instrument trolleys of the labour room might have led to the dissemination of infection during delivery of the babies. Hence, we were able to find a direct correlation of vaginal delivery to the neonatal sepsis. This is similar to the study by Pataskar et al (15). The isolation of environmental strains from the cradle surfaces could have been due to inadequate asepsis (improper hand hygiene and surface cleaning) as BCC can survive in nutrient poor and moist hospital environment (16).

It was noted that all neonate had peripheral IV catheterisation, suggestive of the possible source of dissemination of BCC. Members of BCC are ubiquitous in nature with the potential to survive and multiply in the presence of disinfectants and indwelling invasive medical devices. Hence, they act as a potential reservoir for infections in immunocompromised and hospitalized

patients. BCC is being increasingly recognised as a coloniser of contaminated equipment during hospitalisation (17).

The overall mortality could be limited to four cases by implementation of an effective hospital infection control policy, which included: (i) documentation and communication of outbreak situation to the clinician and administrators (ii) earliest reporting by the microbiologist and prompt response from the clinician (iii) conforming to safe injection practices (iv) enforcing of good infection prevention and control practices like strict adherence to hand hygiene practices, environmental cleaning, use of disinfectants and proper biomedical waste segregation practices (v) conducting competency-based training and monitoring adherence.

Most ICU patients tend to have multiple invasive devices like indwelling IV catheter (peripheral and central), urinary catheter or invasive ventilator. Therefore, we stressed on advising multiple cultures from various sites of any NICU patient immediately after admission and on emergence of new symptoms. The outbreak was successfully controlled with the recovery of 7 out of 11 babies. They improved and were discharged. No new case of BCC was reported in the successive months.

In India various studies on outbreak investigation have been reported till date. In a NICU outbreak reported by Bhise et al. from Nagpur, ten neonates were positive for BCC bacteraemia, however source of infection was not traced (18). Mali et al has reported an outbreak investigation in paediatric patients, with source of BCC being upper surface of rubber stopper of sealed multidose amikacin vials (19).

The present study highlights the role of environmental surveillance in the management of an outbreak investigation of BCC in NICU patients.

Conclusion

The risk of BCC as a common nosocomial pathogen in neonates is significant and warrants careful attention. This highlights the importance of rigorous monitoring, appropriate drug testing, and the establishment of a well-structured hospital infection control policy. This study underscores the proactive and timely intervention of the hospital infection control committee in managing an outbreak. Comprehensive diagnostic efforts and ongoing surveillance are essential to confirm and effectively control such outbreaks.

Acknowledgements

We acknowledge the help of the faculty and technical staff of the Microbiology Department.

Declaration

Not applicable

Ethics approvals

Institutional Ethics Committee (Ref. No. EC/Pharmac/GMC/NGP/4120)

Conflicts of interest

The authors declare that they have no competing interests

Funding source(s)

This study did not receive any grant from funding agencies in the public, commercial, or non-profit organizations.

Author's contributions

Dr. Shayosree Sarkar and Dr. Sonal Chavan - concept, processing, and writing the article
Dr. Geetika Agrawal and Dr Shayosree Sarkar - Sample collection
Dr. Heena Rahangdale - Checked the writing
Dr. Sunanda Zodpey (Shrikhande) – Supervision

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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