



***Burkholderia cepacia* complex: An outbreak in the neonatal intensive care unit of a tertiary care hospital in central India**

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Abstract

Background: *Burkholderia cepacia* complex (BCC) is an opportunistic nosocomial pathogen known to cause severe infections in neonates, including those affecting the respiratory and urinary tracts, as well as bloodstream infections. Consequently, BCC has the potential to instigate outbreaks originating from various sources. This study was undertaken to facilitate the early detection and effective control of a BCC-related outbreak.

Methods: A cross-sectional study, conducted in July 2023 at a tertiary care hospital, investigated bacterial isolates from neonates. Over a one-month period, eleven neonates' blood cultures grew motile, oxidase-positive, non-fermenting gram-negative bacilli. These isolates were presumptively identified as BCC based on conventional biochemical tests and their antimicrobial susceptibility profiles. The recurring isolation of an identical bacterial strain within the neonatal intensive care unit (NICU) strongly suggested an ongoing outbreak. Consequently, active surveillance was initiated to identify the source of the infection and implement containment measures. The identity of the isolated strains was subsequently confirmed using the VITEK 2 Compact microbiology analyser (BioMérieux, France).

Results: Surveillance identified the BCC sources for all 11 affected neonates. The investigation traced the infection origins to several environmental factors within the healthcare setting, including intravenous (IV) catheters and neonatal cradles, operating theatre (OT) beds, and instrument trolleys in the labour room where the deliveries occurred. Furthermore, all environmental BCC isolates demonstrated genetic relatedness to the clinical isolates concurrently exhibiting a similar antibiotic susceptibility pattern. Timely interventions were instrumental in controlling the outbreak.

Conclusion: This study highlights the critical role of the hospital infection control team in effectively managing a BCC outbreak among neonates.

Article Type: Research Article

Article History

Received: 12 June 2024

Received in revised form: 11 February 2025

Accepted: 17 March 2025

Available online: 26 July 2025

DOI: [10.29252/mlj.19.4.14](https://doi.org/10.29252/mlj.19.4.14)

Keywords

[Burkholderia cepacia complex](#)

[Disease outbreaks](#)

[Intensive Care Units](#)

[Sepsis](#)

[Nosocomial](#)



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Introduction

The *Burkholderia cepacia* complex (BCC), a collection of gram-negative, non-fermenting, aerobic betaproteobacteria, was first identified in the 1980s as an opportunistic pathogen capable of causing severe and life-threatening infections in humans. This bacterial group employs a range of virulence mechanisms, contributing to its pathogenicity. These mechanisms include multidrug resistance (Via the *bcrA* efflux pump), genes that determine transmissibility (Such as *esrR* and *cblA*), siderophores (Including salicylic acid, ornibactin, pyochelin, and cepabactin), and adherence proteins (Notably long flexible type II pili) (1,2). These microorganisms exhibit considerable resilience to disinfection and sterilisation methods, demonstrating moderate to high tolerance to various stressors, including ultraviolet (UV)-C radiation, antibiotics, and elevated heavy-metal concentrations. Consequently, developing effective antimicrobial therapies against them presents a significant challenge. The United States Food and Drug Administration (FDA) has, thus, suggested classifying these bacteria as "Objectable Microorganisms" (3). Critically ill neonates in low-resource healthcare settings are at an increased risk of acquiring nosocomial infections within neonatal units. This heightened vulnerability is largely attributed to suboptimal clinical care practices. Neonatal sepsis presents with a range of systemic indicators, such as fever, tachycardia, tachypnoea, and hypotension. A definitive diagnosis also requires the isolation of a pure bacterial culture from at least one anatomical site (4).

This article presents a detailed investigation into a BCC outbreak that affected neonates within a neonatal intensive care unit (NICU) over a one-month period. The successful identification of the outbreak's source was critically dependent on the active involvement of the hospital's

infection control team. Subsequently, timely and precisely targeted interventions, derived from these crucial findings, proved indispensable in effectively containing and controlling the spread of the infection.

Methods

This cross-sectional study, conducted in July 2023 at a tertiary care hospital, investigated 11 neonates referred from the NICU with clinical suspicion of sepsis. All referrals were for blood culture investigations. Biological parameters indicative of infection were recorded, including elevated C-reactive protein (CRP) levels (≥ 6 mg/dL), along with observations of leucocytosis and thrombocytopenia. Blood samples submitted for routine diagnostic testing were processed by the Department of Microbiology at Government Medical College, Nagpur, India. Conventional blood culture bottles (5), specifically five containing brain-heart infusion broth supplemented with 0.025% sodium polyanethol sulfonate, were utilized. Following standard protocols, these blood culture samples underwent sub-culturing onto blood agar, chocolate agar, and MacConkey agar (6). Bacterial cultures grown on blood agar exhibited characteristic smooth, grey, and translucent colonies. Concurrently, non-lactose fermenting (NLF) colonies were observed on MacConkey's agar (Figure 1). Gram-negative isolates demonstrating motility and positive reactions for both catalase and oxidase were subsequently identified to the species level through conventional biochemical testing, as detailed in Figure 2 (6,7). The identification of these isolates was subsequently corroborated using the automated VITEK 2 Compact microbiology analyser (BioMérieux, France). Antimicrobial susceptibility was assessed using both the modified Kirby-Bauer disk diffusion method and the minimum

inhibitory concentration (MIC) by the VITEK 2 antimicrobial susceptibility testing (AST) card. Both methodologies adhered to the guidelines established by the Clinical and Laboratory Standards Institute (2023) (8). All essential media, biochemicals, and disks required for AST procedures were sourced from HiMedia, India.



Figure 1. Non-lactose fermenting translucent moist colonies on MacConkey's agar



Figure 2. Biochemical results: Triple Sugar Iron (TSI): K/K, with no gas or H₂S production; citrate utilized; urea not hydrolyzed; indole negative; methyl red negative; base without any added amino acids, indicating the viability of the organism; ornithine not decarboxylated; lysine decarboxylated; arginine hydrolyzed.

All isolates from blood cultures of 11 NICU-admitted patients consistently showed characteristics of oxidase-positive, motile, non-fermenting gram-negative bacilli (NFGNB). These isolates were further identified as lysine and arginine positive, ornithine negative, and notably resistant to polymyxin B (300 U) and aminoglycosides. The recurrent and sustained detection of these oxidase-positive NFGNB strains strongly suggested a potential outbreak localized within the NICU. Consequently, the presumptive findings were promptly communicated to the intensivists responsible for these patients, and an active surveillance program was initiated and maintained throughout July 2023.

Environmental surveillance was undertaken to identify the origin and transmission pathways of the infection. Samples were systematically collected from a variety of sources, including tap water, intravenous (IV) fluids (Fresh and opened), distilled water designated for humidifiers, water used in patient feeds, and antiseptic solutions. Additionally, pre-moistened sterile swabs were utilized to obtain samples from environmental surfaces, such as medicine trolleys, Ambu bags, and infant cradles (Table 1).

Samples were cultured on blood agar and MacConkey agar plates, which were then incubated at 37°C for 48 hours, as previously described (9). Tap water samples underwent centrifugation at 3000 rpm for 15 minutes, with the resulting sediment subsequently processed. All liquid samples were directly inoculated onto blood agar and MacConkey agar, as well as into brain-heart infusion broth (1:5 dilution). Bacterial plates underwent incubation at 37°C for a 48-hour period. Concurrently, the brain-heart infusion medium was incubated at the same temperature for 5 days, with daily assessments for turbidity (10). If turbidity was observed prior to the fifth day, or on the fifth day itself, subcultures were prepared from the brain-heart infusion and streaked onto blood agar and MacConkey agar plates. Environmental isolates were definitively identified using the automated VITEK 2 Compact microbiology analyser (BioMérieux, France).

For the purposes of this study, an outbreak was rigorously defined as the concurrent identification of more than two patients exhibiting positive culture results for BCC. Outbreak cases specifically referred to neonates who presented with clinical indicators suggestive of sepsis (Including fever, tachycardia, tachypnoea, leukocytosis, or leukopenia, with or without accompanying hypotension) and who also had at least one BCC-positive blood culture.

Table 1. Swabs collected for environmental surveillance

Hospital ward - NICU	
Swabs	Result
Cradle	BCC
Ambu bag	No growth
Intravenous 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	<i>E. coli</i>
Hands of healthcare staff	CoNS

Abbreviations: NICU: Neonatal Intensive Care Unit; BCC: *Burkholderia Cepacia* Complex; Kpn: *Klebsiella pneumoniae*; CoNS: Coagulase Negative *Staphylococcus*; GPB: Gram-Positive Bacilli; *E. coli*: *Escherichia coli*

Results

Eleven neonates with a clinical suspicion of sepsis all tested positive for BCC bacteremia. As detailed in Table 2, the predominant neonatal risk factor linked to this infection was preterm birth (73%), followed by low birth weight (LBW) (36%). The earliest gestational age recorded among the affected neonates was 28 weeks, and the minimum LBW observed was 1.6 kg.

Table 2. Risk factors associated with *Burkholderia cepacia* complex infection

Neonatal risk factors	Number of neonates involved (%)
Preterm	8 (73)
Low birth weight	4 (36)
Hyperbilirubinemia	3 (27)
Respiratory distress syndrome	1 (9)
Multiple organ dysfunction	1 (9)
Congenital anomaly	1 (9)

Environmental monitoring has identified the sources of BCC contamination within the healthcare facility. As detailed in Table 1, these sources include IV catheters and patient cradles in the NICU, operating theatre (OT) tables, and instrument trolleys in the labour room.

Biochemical profiling and AST indicated a close relationship between the bacterial strain under investigation and isolates obtained from neonatal blood cultures. AST further demonstrated that all strains were susceptible to levofloxacin, cotrimoxazole, and minocycline. However, a small number of these strains exhibited resistance to meropenem and ceftazidime.

Discussion

In this study, all 11 neonates who tested positive for BCC in blood cultures were clinically diagnosed with sepsis. These cases presented with diverse pre-existing conditions, including preterm birth, LBW, respiratory distress syndrome (RDS), hyperbilirubinemia, and congenital anomalies (Tetralogy of Fallot). The most prevalent neonatal

risk factors identified were preterm birth (Gestational age <37 weeks), followed by LBW (<2.5 kg), and hyperbilirubinemia. These observations align with findings from previous studies conducted by Belachew et al. (11) and Murthy et al. (12).

Many healthcare professionals administer antibiotics without prior culture of the infection site, which contributes to the nosocomial spread of multidrug-resistant organisms, such as BCC. While previous studies have indicated a higher susceptibility of these organisms to meropenem and ceftazidime (13,14), our study observed a considerably lower susceptibility. Analysis of isolate susceptibility revealed levofloxacin to be the most effective agent, demonstrating superior activity compared to co-trimoxazole and minocycline. Given this resistance profile, levofloxacin was identified as the optimal therapeutic choice. Consequently, following a thorough assessment of its risk-benefit ratio under close medical supervision, levofloxacin therapy was initiated.

Despite therapeutic interventions, all neonates exhibited persistent sepsis without any clinical improvement. Consequently, the antibiotic regimens were modified based on AST results. Following these adjustments, 7 patients demonstrated a positive response to treatment, whereas 4 neonates unfortunately succumbed. Among the fatalities, two were attributed to disseminated intravascular coagulation (DIC), with pre-existing LBW and RDS identified as antecedent contributing factors. The third infant's demise was attributed to septic shock. This individual was diagnosed with acyanotic congenital heart disease. The fourth infant succumbed to sepsis with DIC, with antecedent conditions including hypoxic-ischemic encephalopathy (HIE), preterm birth, and LBW.

Surveillance data indicated that BCC isolated from the surfaces of OT beds and instrument trolleys in the labour room may have contributed to the spread of infection during infant deliveries. Consequently, a direct correlation was identified between vaginal delivery and the occurrence of neonatal sepsis. This finding aligns with the research conducted by Pataskar et al. (15). The presence of environmental strains on the cradle surfaces may be attributed to insufficient aseptic techniques (Such as improper hand hygiene and inadequate surface disinfection), given that BCC is known to persist in nutrient-limited and moist hospital environments (16).

It was observed that every neonate had a peripheral IV catheter, which demonstrates a potential pathway for the dissemination of BCC. BCC members are ubiquitous in the environment and are known for their ability to persist and proliferate even in the presence of disinfectants and on indwelling invasive medical devices. Consequently, they can serve as a significant reservoir for infections, particularly in immunocompromised and hospitalized patients. There is growing recognition of BCC as a colonizer of contaminated medical equipment during hospitalization (17).

The total mortality rate could be reduced to just four cases through the implementation of a robust hospital infection control policy, encompassing (i) thorough documentation and clear communication of the outbreak status to both clinicians and administrators; (ii) rapid reporting by microbiologists, coupled with immediate responses from clinicians; (iii) strict adherence to safe injection practices; (iv) rigorous enforcement of infection prevention and control measures, including meticulous hand hygiene, comprehensive environmental cleaning, appropriate disinfectant use, and proper segregation of biomedical waste; and (v) regular competency-based training alongside continuous monitoring of adherence to these guidelines.

NICU-admitted patients frequently require multiple invasive medical devices, including peripheral and central IV catheters, urinary catheters, and invasive mechanical ventilation. Consequently, we advocate for obtaining multiple cultures from various anatomical sites of any NICU patient immediately upon admission and whenever new symptoms emerge. This approach successfully controlled the outbreak, with 7 out of 11 infants recovering and being discharged. No new cases of BCC were reported in the subsequent months.

Several studies on outbreak investigations have been documented in India. For instance, Bhise et al. reported a NICU outbreak in Nagpur, where 10 neonates tested positive for BCC bacteremia; however, the infection source remained unidentified (18). In another instance, Mali et al. detailed an outbreak investigation among pediatric patients, where the source of BCC was traced to the upper surface of rubber stoppers on sealed multidose amikacin vials (19).

This study underscores the critical role of environmental surveillance in effectively managing outbreaks of BCC among NICU-admitted patients.

Conclusion

The considerable risk of BCC as a prevalent nosocomial pathogen in neonates necessitates close scrutiny. This emphasizes the critical need for stringent monitoring, proper drug testing, and the implementation of a robust hospital infection control policy. The present research highlights the critical role of prompt and timely intervention by a hospital's infection control committee during an outbreak. It emphasizes that comprehensive diagnostic efforts and ongoing surveillance are indispensable for both confirming and effectively managing such events.

Acknowledgement

We would like to thank the faculty members and technical staff of the Microbiology Department for their invaluable assistance.

Funding sources

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

Ethical statement

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

Conflicts of interest

No conflicts of interest.

Author contributions

Dr. Shayosree Sarkar and Dr. Sonal Chavan: Conceptualization, Methodology, and Manuscript preparation. Dr. Geetika Agrawal and Dr. Shayosree Sarkar: Sample acquisition. Dr. Heena Rahangdale: Critical review and Editing of the manuscript. Dr. Sunanda Zodpey (Shrikhande): Project supervision.

Data availability statement

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

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Cite this article as:

Sarkar S, Chavan S, Agrawal G, Rahangdale H, Zodpey S. *Burkholderia cepacia* complex: An outbreak in the neonatal intensive care unit of a tertiary care hospital in central India. *Med Lab J*. 2025;19(4):14-7. <http://dx.doi.org/10.29252/mlj.19.4.14>