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Outbreak of *Burkholderia gladioli* septicemia in a neonatal intensive care unit: Investigation and control measures

Karvi Agarwal [1] , Amit Garg [1] , Konpal Agarwal [2] , Naila Begum [1]*

1. Department of Microbiology, LLRM Medical College, Meerut, Up, India
2. Department of Pathology, Subharti Medical College, Meerut, Up, India
* Correspondence: Naila Begum. Department of Microbiology, LLRM Medical College, Meerut, Up, India.
Tel: +918853427680; Email: naila.1668@gmail.com

Abstract

Background: *Burkholderia gladioli* (*B. gladioli*) is a rare but emerging pathogen associated with neonatal sepsis. This case series describes the investigation and control of an outbreak in a tertiary neonatal intensive care unit (NICU) in India.

Methods: In January 2024, seven neonates admitted to the NICU developed bloodstream infections. Clinical manifestations included respiratory distress, feeding refusal, metabolic acidosis, seizures, and shock. Predisposing factors included prematurity, congenital anomalies, and perinatal asphyxia. Blood cultures were processed using the automated BD BactecFX-40 system, and organism identification with antimicrobial susceptibility testing was performed using the BD Phoenix M-50 system. Environmental sampling was conducted to identify the outbreak source.

Results: Of 72 blood culture bottles, 34 (47.2%) flagged positive. *B. gladioli* were isolated from seven patients, accounting for 20.6% of positive cultures. The overall mortality rate was 57.1% (4/7 Deaths). All clinical isolates showed identical susceptibility patterns, with susceptibility to levofloxacin, cotrimoxazole, ceftazidime, chloramphenicol, minocycline, and meropenem, and intrinsic resistance to colistin. Environmental surveillance identified the same *B. gladioli* strain from suction apparatus and phototherapy units. Contributory factors included poor hand hygiene compliance (28-33%), limited glove availability, and overcrowding. Following cohorting, deep cleaning with 1% hypochlorite, staff retraining, and reinforcement of infection control protocols, no further isolates were recovered.

Conclusion: This outbreak highlights the high mortality associated with *B. gladioli* sepsis in neonates and underscores the critical need for stringent infection control practices and automated identification systems in NICUs. To our knowledge, this is the first such report from India.

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Introduction

Burkholderia gladioli (*B. gladioli*), formerly known as *Pseudomonas marginata*, is a recognized plant pathogen; however, human infections caused by this bacterium have rarely been reported. It is an aerobic, motile, non-spore-forming, catalase-producing, non-lactose-fermenting, Gram-negative bacillus that is widely distributed in the environment (1).

Over the past two decades, *B. gladioli* have emerged as a clinically significant human pathogen, causing severe necrotizing pneumonia and neonatal bacteremia, including both early-onset and nosocomial sepsis in newborns (2). The primary diagnoses reported among affected neonates include severe major congenital anomalies, prematurity with respiratory distress syndrome, pneumonia, and parapneumonic pleural effusion (1).

It also causes superficial, deep-seated, and disseminated infections, including meningitis, peritonitis, septicemia, and bronchiectasis (3). It frequently colonizes the lungs of patients with cystic fibrosis (CF) and has emerged as a significant opportunistic pathogen in immunocompromised and hospitalized patients, as well as in patients with chronic granulomatous disease (CGD) (3).

Nosocomial transmission of this organism is facilitated by cross-transmission, frequent pulmonary procedures, and central venous access, and multiple outbreaks in newborns have been documented (4). The overall in-hospital mortality rate has been reported as 21.4%, whereas the global mortality rate is approximately 7% (1).

B. gladioli have been isolated from wet surfaces, where it can survive and persist for prolonged periods, including in disinfectants and intravenous fluids (5). Hospital outbreaks are frequent and are usually linked to a single contaminated source, such as drinking water, distilled water, disinfectants, intravenous solutions, multidose antibiotic vials, nebulizer solutions, flow meters, nasal sprays, ultrasound gels, anesthetics, suction pumps, phototherapy units, and respiratory therapy equipment (6).

The high transmissibility of *B. gladioli* in hospital settings, together with its intrinsic resistance to several antibiotics and poor prognosis, highlights the importance of early detection and prompt treatment of *B. gladioli* infections (7).

In this article, we present our experience in investigating and managing an outbreak of nosocomial *B. gladioli* sepsis transmission in the neonatal intensive care unit (NICU) of Government Medical College and its associated hospital in Meerut city, Western Uttar Pradesh, India.

To the best of our knowledge, this is the first study from India, particularly from Western Uttar Pradesh, making it highly important for increasing awareness of this unusual pathogen in healthcare settings.

Methods

During January 2024, seven blood cultures received in our clinical microbiology laboratory showed morphologically similar bacterial growth. On further investigation, all samples were traced to neonates admitted to our NICU. The clinical microbiologist alerted the concerned HIC team, the head pediatrician, and the hospital administration.

Because an outbreak was suspected, the NICU in-charge and HIC teams were activated to follow up the patients and investigate the outbreak for corrective and preventive actions (CAPA) and root cause analysis (RCA). The mortality rate for this outbreak was very high at 57.1%.

Environmental surveillance was conducted in the NICU. Surface swabs were collected from multiple NICU surfaces, including phototherapy units, suction apparatus, laryngoscopes, the crash cart trolley, and the instrument trolley. All samples were sent to the microbiology laboratory for aerobic culture and sensitivity testing. *B. gladioli* were isolated from surface swabs collected from the suction apparatus and phototherapy units. The antimicrobial susceptibility testing (AST) pattern was similar to that observed in the patients' blood cultures. Meanwhile, all neonates were temporarily shifted to another

ICU area until NICU disinfection measures had been completed. All patient details were anonymized, coded by randomization, and delinked from patient identity.

The case histories and outcomes of the seven neonates in the NICU with positive blood cultures for *B. gladioli* are presented below.

Case No. 1

A single term (38 Weeks of gestation), appropriate-for-gestational-age (AGA), 2.53 kg Caucasian female baby was delivered by lower segment cesarean section (LSCS) at our hospital. The baby cried immediately after birth, with APGAR scores of 6 and 8 at 1 and 5 minutes, respectively. Subsequently, the baby developed refusal to feed a few hours after birth. A blood culture was sent to the clinical microbiology laboratory on day 3 after delivery, and the baby was started on intravenous cefotaxime (100 mg in 10 ml of normal saline twice per day) and amikacin (15 mg every 24 hours). The blood culture showed positive microbial growth of *B. gladioli* 72hr after it was sent, and antibiotic treatment was changed to injectable meropenem. The patient improved and was discharged on day 14.

Case No. 2

A preterm (31 Weeks of gestation), extremely low-birth-weight (1.4 kg) Caucasian male baby was delivered by LSCS to a primigravida at our hospital. The baby was admitted to the NICU of our hospital on day 1 of life. The mother had a history of leaking per vaginum (PV) for 2 months and was receiving antibiotics. She also had an antenatal history of infection with rubella and cytomegalovirus. The baby cried immediately after birth but later showed signs of respiratory distress, including nasal flaring, chest retractions, and a respiratory rate of 48 breaths/minute. The baby was treated with continuous positive airway pressure (CPAP). Intravenous cefotaxime and amikacin were started. A blood culture sent to the clinical microbiology laboratory on the 5th day of admission showed microbial growth of *B. gladioli*, and intravenous meropenem was added to amikacin. The patient improved and was discharged on day 17 of admission.

Case No. 3

A single, post-term (43 Weeks of gestation), AGA, 3.52 kg Caucasian male baby was born to a primigravida woman. The mother reported a history of pre-eclampsia during the antenatal period and had obstructed labor. The baby was delivered by normal vaginal delivery (NVD) at our hospital. The baby did not cry immediately after birth and had meconium-stained liquor with signs of fetal distress and perinatal asphyxia. On the first day of life, he was admitted to the NICU and started on CPAP therapy. A blood culture was sent on day two of admission. The blood culture sent to the clinical microbiology laboratory became positive on day 3 of life, with microbial growth of *B. gladioli* reported on day 4 after the blood culture was sent. The baby was initially started on intravenous cefotaxime (100 mg in 10 ml of normal saline twice per day) and amikacin (15 mg every 24 hours). The baby's clinical condition did not improve, and he expired on the 7th day of admission.

Case No. 4

A single term (36 Weeks of gestation), AGA, 2.13 kg Caucasian male baby was born to a multigravida woman. The baby was delivered by LSCS due to breech presentation at our hospital. The mother had a history of stillbirth during her previous NVD. The baby had complaints of loose watery stools for 1 day, followed by low-grade fever for 1 day. Ultrasonography (USG) of the cranium performed on the 24th day of life revealed a left-sided choroid plexus cyst. The blood culture sent to the clinical microbiology laboratory on day 1 after delivery revealed microbial growth of *B. gladioli* after 48hrs of blood culture. Injection amikacin was given initially and was supplemented with meropenem after the diagnosis of septicemia. The patient died on day 5 after delivery.

Case No. 5

A single, term, AGA, 3.1 kg female baby was delivered by LSCS to a multigravida woman. The mother had a history of leaking per vagina and dai handling during her current delivery. The baby was admitted to the NICU with a history of ruptured meningo-omphalocele, congenital hydrocephalus, and bilateral iliac fossa cysts. USG of the cranium revealed a dilated ventricular system and a choroid plexus cyst on the left side. A blood culture sent to the microbiology department on day 1 showed positive growth of *B. gladioli* on day 4 after the blood culture was sent. The baby was receiving injection amikacin and injection cefotaxime. The baby did not show any signs of improvement and expired on day 3 after delivery, before the blood culture report was available.

Case No. 6

A single term (36 Weeks of gestation), 2.7 kg Caucasian female baby was delivered by normal vaginal delivery to a multigravida woman at our hospital. The mother reported a history of oligohydramnios with premature rupture of membranes for 16 days. The baby was admitted to the NICU on day 1 of life. The baby did not cry immediately after birth and was admitted with metabolic acidosis, seizures, shock, and sepsis. On day 3 of life, the baby was shifted to mechanical ventilation. Empirical treatment with intravenous cefotaxime (90 mg in 10 ml of normal saline twice per day) and amikacin (32 mg every 36 hours) was started. Blood culture showed microbial growth of *B. gladioli* on day 4, and antibiotic treatment was changed to injectable meropenem. The baby came off ventilator support on day 8 (After 72 hrs of injection meropenem). The baby improved and was discharged on day 21 after delivery.

Case No. 7

A single, preterm (32 Weeks of gestation), 1.5 kg Caucasian female baby born to a primigravida by LSCS delivery was admitted to the NICU on day 1 of life. The mother reported a history of placenta previa during the antenatal period. The baby presented with severe anemia and a pansystolic murmur. She subsequently developed respiratory distress and was placed on mechanical ventilation on day 2 after delivery. A blood culture sent on day 3 showed growth of *B. gladioli*. The baby was started on injection meropenem but did not show any signs of improvement and was declared dead on day 11.

Microbiological analysis

All blood culture samples were collected in BD BactecFX-40 (Becton Dickinson) aerobic blood culture bottles and sent to the hospital's clinical microbiology laboratory. The samples were incubated and monitored regularly using the automated system. Bottles that flagged positive were removed from the instrument, Gram-stained, and subculture on 5% sheep blood agar (SBA) and MacConkey's (MAC) agar plates. On SBA, growth appeared as typical large, circular, low-convex, opaque, glistening, initially non-pigmented colonies that later developed yellowish pigmentation (β-hemolytic), with non-lactose-fermenting (NLF) colonies on MAC agar. Gram staining of the culture smear revealed Gram-negative bacilli. The isolate was catalase-positive and weakly oxidase-positive. Final identification and antibiotic susceptibility testing were performed using the automated BD Phoenix M-50 (Becton Dickinson) system. Results were interpreted as MIC values for each drug. The isolates were identified as *B. gladioli* with similar antimicrobial susceptibility patterns (Table 1).

Surveillance cultures

NICU surveillance samples were collected using sterile swabs and sent immediately to our hospital's clinical microbiology laboratory. Samples were taken from ventilator tubes, suction apparatus, phototherapy units, Ambu bags, Cheatle forceps, injection preparation areas, vials, taps, bed rails, sterile saline used for injection preparation, humidifiers, warmers, oxygen tubing and masks, laryngoscope blades, medicine trays, and pulse oximeters. Swabs were plated on SBA and MAC agar plates and incubated overnight at 36 ± 1°C under aerobic conditions. The plates were read the following day, and colonies were identified using Gram staining and biochemical tests.

The BD Phoenix system (Becton Dickinson) was used for identification and antimicrobial susceptibility testing of these isolates. *B. gladioli* were isolated from surveillance samples collected from suction apparatus and phototherapy units. These cases clustered within a very short period, indicating direct access of this pathogen to the bloodstream and causing a neonatal septicemia outbreak. All eight isolates (Clinical = 7 and Surveillance = 1) were identical based on similar susceptibility to a group of drugs, namely levofloxacin, cotrimoxazole, ceftazidime, chloramphenicol, and minocycline.

After drug susceptibility results became available for this bacterium, the treatment of the babies was changed from injection cefotaxime to injection meropenem, with the expectation of a better clinical outcome. Unfortunately, mortality remained high in these cases because of other predisposing factors.

Table 1. Antimicrobial susceptibility pattern of isolates

Isolate	Levofloxacin		Cotrimoxazole		Ceftazidime		Chloramphenicol		Minocycline		Meropenem		Colistin	
	MIC	Interpretation	MIC	Interpretation	MIC	Interpretation	MIC	Interpretation	MIC	Interpretation	MIC	Interpretation	MIC	Interpretation
1	≤ 0.5	S	≤ 20	S	≤ 1	S	≤ 4	S	≤ 2	S	≤ 0.25	S	≥ 32	IR
2	≤ 1	S	≤ 20	S	≤ 0.5	S	≤ 2	S	≤ 4	S	≤ 0.25	S	≥ 8	IR
3	≤ 0.5	S	≤ 20	S	≤ 1	S	≤ 2	S	≤ 2	S	≤ 0.25	S	≥ 16	IR
4	≤ 1	S	≤ 20	S	≤ 1	S	≤ 2	S	≤ 2	S	≤ 0.25	S	≥ 16	IR
5	≤ 1	S	≤ 20	S	≤ 0.25	S	≤ 4	S	≤ 4	S	≤ 0.25	S	≥ 32	IR
6	≤ 0.5	S	≤ 20	S	≤ 1	S	≤ 4	S	≤ 4	S	≤ 0.25	S	≥ 32	IR
7	≤ 0.5	S	≤ 20	S	≤ 1	S	≤ 2	S	≤ 4	S	≤ 0.25	S	≥ 32	IR

*MIC: Minimum Inhibition Concentration (µg/ml), S: Susceptible, R: Resistant, IR: Intrinsically Resistant

Complete disinfection measures were implemented in the NICU to break the chain of transmission. Suction apparatus jars and phototherapy units were thoroughly scrubbed with detergent and hot water, completely dried, and then decontaminated with 1% hypochlorite solution. Daily replacement of suction jars and replacement between patients were initiated according to infection prevention protocol practices. Daily disinfection practices were implemented and strictly monitored in the ICU by the HIC team.

Retraining on hand hygiene, cleaning, and disinfection procedures was conducted for NICU staff. After CAPA measures were implemented, environmental surveillance was repeated, and this bacterial pathogen was not isolated again.

Results

During the outbreak period in January 2024, a total of 72 blood culture bottles were received in the Microbiology Department of LLRM Medical College and the associated SVBP Hospital for automated blood culture and sensitivity testing.

Of the 72 bottles, 34 signaled/flagged positive for microbial growth, with a positivity rate of 47.2%. Among the positive cultures, *Escherichia coli* was isolated in 10 (29.4%), followed by *B. gladioli* in 7 (20.6%), *Staphylococcus aureus* and *Klebsiella* spp. in 5 (14.70%) each, *Proteus* spp. in 4 (11.76%) cases, and coagulase-negative *Staphylococcus* spp. in 3 (8.82%) cases.

It should be noted that *B. gladioli* were isolated from seven different patients, contributing a substantial proportion of 20.6% of total positive cultures.

The female-to-male gender distribution ratio was 5:2.

Identification and antimicrobial susceptibility testing were performed using the automated BD Phoenix system. MIC values of the drugs were reported with interpretations (Table 1).

The antimicrobial susceptibility patterns of all isolates were almost identical.

B. gladioli showed intrinsic resistance to the polymyxin class of antibiotics (Colistin sulphate).

Discussion

According to the hospital infection control policy, an outbreak is considered when a similar microbial infection is isolated from two or more patients within a defined period of time and the antibiotic susceptibility pattern is comparable. For this study, a neonate with clinical suspicion of sepsis and two or more *B. gladioli*-positive blood culture results was considered part of the outbreak. The outbreak was suspected in January 2024 in the NICU, and an investigation was triggered when seven subsequent cases of bacteremia caused by *B. gladioli* occurred within a period of 1 month. This prompted detailed microbiological investigation and monitoring of hospital infection surveillance activities in the NICU. RCA performed by the HIC team traced the source to suction apparatus and phototherapy units, resulting from a breach in strict infection control practices, for which CAPA was implemented.

Low hand hygiene compliance rates (28% for doctors and 33% for nurses), limited availability of gloves for handling staff, and overcrowding due to patients' visitors inside the NICU were identified as supportive factors for this outbreak (8).

Retraining sessions on hand hygiene were conducted by the HIC team, and regular monitoring was performed to improve compliance rates. Hand rubs, gloves, and PPE were made available to NICU staff. Entry was limited to restricted individuals under strict infection control protocols. Visitors were informed and trained regarding the importance of hand hygiene practices through pamphlets displaying the same information.

In previous studies, the predominant age groups were adults and children (9,10). Boyanton et al. (11) suggested that *B. gladioli* is likely underreported by clinical microbiology laboratories because of its fastidious nature and the difficulty of isolating it by conventional methods without automation.

Four neonates died during hospitalization, resulting in a very high mortality rate of 57.1%. This was attributed to additional associated and predisposing factors, including perinatal asphyxia, congenital hydrocephalus, choroid plexus cyst, respiratory distress with cardiac involvement, and ruptured meningo-omphalocele. Our study showed the highest mortality rate, 57.1%, compared with the study by Dursun et al. (1), which reported 7% in Turkey.

Conclusion

This outbreak was an eye-opener for the entire health system. The lack of data from our geographic area prompted us to report this outbreak and raise awareness of this bacterium as an "Alarming peril" in critical healthcare settings.

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Ethical Statement

Institutional Ethics Committee - Ref. No. SC-1/2024/1431

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Author Contributions

Dr. Karvi Agarwal designed the study and revised the manuscript for important intellectual content. Dr. Amit Garg provided critical review for manuscript revision. Dr. Konpal Agarwal collected and analyzed the data. Dr. Utkarsh Khattri provided critical inputs. Dr. Karvi Agarwal and Dr. Naila Begum performed the laboratory work. The final manuscript was approved by all authors.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its supplementary information files.

Use of Artificial Intelligence

The authors declare that no artificial intelligence, large language models, or generative AI tools were used in the conception, research, data analysis, or drafting of this manuscript.

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