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

Running Title: *Burkholderia gladioli* septicemia outbreak in a NICU

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Abstract

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

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

Results: Out of 72 blood culture bottles, 34 (47.2%) flagged positive. *B. gladioli* was isolated from seven patients (20.6% of positive cultures). The overall mortality rate was 57.1% (4/7 deaths). All clinical isolates demonstrated identical susceptibility patterns: sensitive to levofloxacin, cotrimoxazole, ceftazidime, chloramphenicol, minocycline, and meropenem, with 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. After implementing cohorting, deep cleaning with 1% hypochlorite, staff retraining, and reinforcing infection control protocols, no further isolates were recovered.

Conclusion: This outbreak highlights the high mortality of *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.

Keywords: *Burkholderia gladioli, Pseudomonas marginata,* Outbreak, Septicemia, NICU, Antimicrobial susceptibility pattern

Introduction

Burkholderia gladioli (*B. gladioli*), formerly known as *Pseudomonas marginata*, is a known plant pathogen, but human infections due to these bacteria have rarely been reported. It is an aerobic, motile, non-spore forming, catalase producing, non- lactose fermenting, Gram-negative bacillus widely distributed in the environment (1).

It has become a significant human pathogen in the last two decades, producing lethal necrotizing pneumonia and neonatal bacteremia, causing early and nosocomial sepsis in newborns (2). Primary diagnoses in the neonates were severe major congenital anomalies, prematurity with respiratory distress syndrome, pneumonia, and para-pneumonic pleural effusion (1).

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

The nosocomial spread of this organism is aided by cross-transmission, frequent pulmonary operations, and central venous access, and many outbreaks in newborns have been documented (4). The overall in-hospital mortality rate was 21.4 %, whereas the mortality rate was approximately 7 % worldwide (1).

B. gladioli has been isolated from wet surfaces where it survives and persists for long periods, including disinfectants and intravenous fluids (5). Hospital outbreaks are frequent and are usually due to a single contaminated source, such as drinking water, distilled water, disinfectants, intravenous solutions, multi-dose 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* infection in hospitals, intrinsic resistance to several antibiotics, poor prognosis highlights the importance of early detection and treatment of *B. gladioli* infections (7).

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

To the best of our knowledge, this is the first study from India and of Western Uttar Pradesh state which makes it utmost important for spreading awareness about this unusual pathogen among the healthcare settings.

Cases presentation

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

An outbreak was suspected, so the NICU in-charge and HIC teams were activated to follow up the patients and to investigate this outbreak for CAPA and RCA. The mortality rate for this outbreak was found to be very high that was 57.1%.

Environmental surveillance was conducted in the NICU. Surface Swabs were collected from various surfaces of NICU like phototherapy units, suction apparatus, Laryngoscope, crash cart trolley, instrument trolley etc. All samples were sent for Aerobic Culture and Sensitivity in Microbiology laboratory. *B.gladioli* was isolated from the surface swabs of Suction apparatus and phototherapy units. A similar AST pattern was observed as reported from the patient's blood culture. Meanwhile all the babies were temporarily shifted to other ICUs space till disinfection measures completed for NICU space. All patient details were anonymized, coded by randomization, and delinked from any identity of the patients.

The following are the case history and outcome of the seven neonates in NICU with positive Blood culture for *B.gladioli.*

Case No. 1

A single term (38 weeks of gestation), appropriate for gestational age (AGA) with birth-weight (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 score of 6 & 8 at 1 and 5 minutes respectively. Subsequently, the baby showed a refusal to feed a few hours after birth. A blood culture was sent to the clinical microbiology laboratory on day 3 of delivery, and the baby was started on intravenous injections of Cefotaxime (100 mg in 10 ml of normal saline twice per day) and Amikacin (15 mg every 24 hours). A blood culture shows a positive microbial growth of *B.gladioli* after 72hr of Blood culture 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 (1.4kg) Caucasian male baby was delivered by a primigravida by LSCS at our hospital. The baby was admitted to the NICU of our hospital on day 1 of life. The mother had history of leaking Per vaginum (PV) for 2 months and was receiving antibiotics. She had antenatal history of infection with Rubella and Cytomegalovirus. The baby cried immediately after birth, but later showed signs of respiratory distress (nasal flaring, chest retractions, respiratory rate of 48 breaths/minute). The baby was treated with continuous positive airway pressure (CPAP). Baby was started on intravenous injections of Cefotaxime and Amikacin. A blood culture was sent to clinical microbiology laboratory on the 5th day of admission, shows a microbial growth of *B.gladioli* and I.V. Injections of Meropenem were added with 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 born to a primigravida female. 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 his first day of life, he was admitted in NICU and began receiving CPAP therapy. A blood culture was sent on day two of admission. His blood culture sent to the clinical microbiology laboratory turned positive on day 3 of life with a microbial growth of *B.gladioli* on day 4 of sending blood culture. The baby was started on intravenous injections of cefotaxime (100 mg in 10 ml of normal saline twice per day) and amikacin (15 mg every 24 hours) initially. The baby's clinical condition did not improve and was expired on 7th day of admission

Case No. 4

A single term (36 weeks of gestation), AGA, 2.13kg Caucasian male baby born to multi-gravida female. The baby was delivered by LSCS due to breech presentation at our hospital. The mother had a history of still birth during her previous NVD. The baby had complaints of loose watery stools for 1 day which is followed by low grade fever for 1 day. USG of the cranium was done on 24th day of life revealed choroid plexus cyst of left side. The blood culture sent to the clinical microbiology laboratory on day 1 of delivery, revealed a microbial growth of *B.gladioli* after 48hrs of blood culture. Injection Amikacin given initially added with Meropenem after diagnosis with septicemia. The patient died on day 5 of delivery.

Case No.5

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

Case No. 6

A single term (36 weeks of gestation), 2.7kg Caucasian female baby was delivered by a multigravida female by normal vaginal delivery at our hospital. The mother reported a history of oligohydramnios with premature

rupture of the membranes for 16 days. The baby was admitted in the NICU on day 1 of life. The baby did not cry immediately after birth and was admitted with the complaints of metabolic acidosis, seizures, and shock with sepsis. On day 3 of life, the baby was shifted to mechanical ventilation. Empirical treatment with intravenous injections of cefotaxime (90 mg in 10 ml of normal saline twice per day) and amikacin (32 mg every 36 hours) was started. Blood culture showed a microbial growth of *B.gladioli* on day 4, and antibiotic treatment was changed to injectable Meropenem. Baby came out of ventilator support on day 8 (after 72hrs of injection Meropenem). Baby improved and was discharged on day 21 of delivery.

Case No. 7

A single, preterm (32 weeks of gestation), 1.5kg 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 pan-systolic murmur. He subsequently developed respiratory distress and was put on mechanical ventilation on day 2 of delivery. Blood culture sent on day 3, showed a growth of *B.gladioli*. The baby was started on injection Meropenem, but baby did not show any signs of improvement and was declared dead on day 11.

Microbiological analysis

All the blood culture samples were collected in BD BactecFX-40 (*Becton Dickinson*) aerobic blood culture bottles and were sent to the hospital's clinical microbiology laboratory. The samples were incubated and monitored regularly using the automated system. The bottles which flagged positive were removed from the instrument, Gram-stained, and sub-cultured on 5% Sheep blood agar (SBA) and MacConkey's (MAC) agar plates. On SBA, the growth was observed as typical large, circular, low-convex, opaque, glistening, non-pigmented initially, later developing yellowish pigmentation (β-hemolytic) colonies and non-lactose-fermenting (NLF) colonies on MAC agar. Gram's stain was performed on culture smear, which revealed Gram negative bacilli. It was both catalase and weak oxidase-positive. The final identification and antibiotic susceptibility of the bacteria was done with the help of automated BD-Phoenix M-50 (*Becton Dickinson*) system. The results were interpreted in the form of MIC values for the drug. They were identified as *B.gladioli* with similar antimicrobial susceptibility pattern (Table 1).

Surveillance cultures

NICU surveillance samples were collected with the help of 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, and sterile saline for injection preparations, humidifiers, warmers, Oxygen tubings and mask, Laryngoscope blade, medicine trays, Pulse oximeter. Swabs were plated on SBA and MAC agar plates and incubated overnight at 36 ± 1°C under aerobic conditions. The plates were read next day, the colonies were identified using Gram's staining and biochemical tests.

BD Phoenix system (Becton Dickinson) was used for identification and antimicrobial susceptibility of these isolates. *B.gladioli* were isolated from surveillance samples of a suction apparatus and phototherapy units. These cases clustered in a very short period pointing to direct access of this pathogen to the blood stream causing neonatal septicemia outbreak. All eight isolates (clinical =7 and surveillance = 1) were found to be identical based on similar susceptibility to group of drugs namely Levofloxacin, Cotrimoxazole, Ceftazidime, Chloramphenicol, and Minocycline.

After knowing the drug susceptibility for this bacterium, the treatment of babies was changed from Inj. Cefotaxime to Inj. Meropenem, expecting better clinical outcome, but unfortunately the mortality was high in these cases due to other predisposing factors.

Complete Disinfection measures were taken for NICU to break the chain of transmission. Suction apparatus jars and phototherapy units were cleaned with thorough scrubbing with detergent and hot water, dried completely, followed by decontamination with 1% hypochlorite solution. Daily change of suction jars and change in between each patient were initiated as per infection preventive protocol practices. Daily disinfection practices were implemented and monitored as a strict protocol in ICU by the HIC team.

Retraining on hand hygiene, cleaning, and disinfection procedures was conducted for NICU staff. After the CAPA measures, Environmental surveillance was redone, on which this bacterial pathogen was not isolated again.

Results

During the outbreak period of 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.

Out of 72, 34 bottles 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%) followed by 5 (14.70%) each of *Staphylococcus aureus* and *Klebsiella* spp. *Proteus* spp. was isolated in 4(11.76%) cases. However, coagulase-negative *Staphylococcus* spp. were isolated in 3 (8.82%) cases.

It is to be noted that *B. gladioli* were isolated from seven different patients, contributing to a major percentage of 20.6% of total positivity.

The Gender distribution ratio of female to male was 5:2

The Identification and Antimicrobial susceptibility testing were performed by the Automated BD Phoenix system. MIC values of the drugs were reported with interpretation (Table 1).

The antimicrobial susceptibility pattern of all isolates was almost similar.

B. gladioli shows intrinsic resistance to the polymyxin class (colistin sulphate) of antibiotics.

Discussion

An outbreak is considered when a similar microbial infection is isolated from two or more patients in a defined period of time and the antibiotic susceptibility pattern is comparable, according to the hospital infection control policy. A neonate with a clinical suspicion of sepsis and two or more *B. gladioli* positive blood culture results was considered as an outbreak for this study. The outbreak was suspected in January 2024 in 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 us for detailed microbiological investigation and to monitor hospital infection surveillance activities in NICU. On RCA done by HIC team, source was traced to be from the suction apparatus and phototherapy units due to breach in strict infection control activities, for which CAPA done.

Low hand hygiene compliance rates (28% for doctors and 33% for nurses), limited availability of the gloves to the handling staff, overcrowding with the patient's visitors inside the NICU was found as supportive factors for this outbreak (8).

Retraining sessions on hand hygiene was conducted by the HIC team and regular monitoring was done to increase the compliance rate. Hand-rubs, gloves and PPE was made available to the staff of NICU. The entry was made limited to the restricted individuals with strict infection control protocols. The visitors were explained and trained about the importance of hand hygiene practices in the form of a pamphlet displaying the same.

In all previous studies predominant age group were adults and children (9,10) Boyanton *et al*. (11) suggested that *B.gladioli* is most likely under reported by clinical microbiology laboratories due to its fastidious nature and difficulty isolating it without automation from conventional means.

Four neonates died while being hospitalized causing very high mortality 57.1%. This was due to additional associated and predisposing factors like perinatal asphyxia, congenital hydrocephalous, choroid plexus cyst, respiratory distress with cardiac involvement, ruptured meningo-omphalocele. Our study shows the highest mortality rate 57.1 % as compared to studies by Darsun*et al*.,[1]reported 7% in Turkey.

Conclusion

This outbreak was an eye opener to the entire health system. Lack of data in our geographic area prompted us to pen it down, to bring awareness regarding this bacterium as "alarming peril" in critical health settings.

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Ethical Statement

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

Conflict of Interest

There are no conflicts of interest to declare by any of the authors.

Authors Contribution

Dr. Karvi Agarwal: Designed The Study and Revised the Manuscript for Important Intellectual Content. Dr. Amit Garg: Provided Critical Review for Revision of Manuscript. Dr. Konpal Agarwal: Collected and Analyzed the Data. Dr. Utkarsh Khattri: Provided the Critical Inputs. Dr. Karvi Agarwal and Dr. Naila Begum: Performed the Laboratory Work. The final manuscript was approved by all authors.

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Table 1: Antimicrobial susceptibility pattern of isolates

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