An Outbreak of Burkholderia Cepacia Septicemia in Neonatal Intensive Care Unit of a Tertiary Care Hospital, Peshawar

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Abstract

Background: *Burkholderia cepacia* (formerly *Pseudomonas*) is a gram-negative bacillus that can remain viable in low-nutrient water and is typically found in soil and moist settings. It is one of the leading causes of sepsis in infants, and it is spread by human contact with contaminated medical devices and disinfectants. *B. cepacia* has emerged as a significant opportunistic pathogen in hospitalized and immunocompromised patients, colonizing the lungs of individuals with cystic fibrosis. Hospital outbreaks have been associated to infected faucets, nebulizers, disinfection solutions, multi-dose antibiotic vials, drinking water, distilled water, flowmeters, nasal sprays, and ultrasound gels. We describe our investigation and successful management of a nosocomial *Burkholderia cepacia* sepsis outbreak in a tertiary care hospital's newborn intensive care unit in Peshawar, Pakistan.

Methodology: Blood samples from 50 newborns with sepsis were collected using a standardized approach and incubated using an automated blood culture system (BACT/Alert 3D and BACTEC 9050). Disk diffusion and the Minimum inhibitory concentration methods were used to test antimicrobial susceptibility. Gram staining was used to identify bacteria, and API (Analytical Profile Index) 20 NE was used to characterize them biochemically. Environmental and epidemiological investigations were also conduct to investigate the source and route of infection.

Results: All of the 50 patients admitted in NICU (Neonatal Intensive Care Unit) were included in this study, and had lately-onset neonatal sepsis, with *B. cepacia*. During an epidemic in the NICU from 30th, April to 21st, June 2021, *B. cepacia* was isolated from over 45 blood samples. In total 45 neonates 17(35%) were female and 28 (65%) were male. Average age of neonates was 14.88 days. Antibiotic susceptibility testing was performed on Muller Hinton agar using Kirby Bauer’s disc diffusion method and interpreted according to Clinical Laboratory Standard Institute (CLSI 2021) guidelines. As *B. cepacia* has intrinsic resistant to polymyxin class (colistin sulphate) of antibiotics, the susceptibility pattern of all isolates were almost similar i.e. showing resistance to tetracycline 100% (minocycline) and cephalosporin 3rd generation 100% (ceftazidime). The isolates were 100% sensitive to fluoroquinolones (levofloxacin), carbapenem 100% (meropenem), chloramphenicol 100% and sulfonamide 100% (co trimaxazole).

Conclusion: In present study, the index case might have been exposed to infection due to an intravenous fluid utilized for fluid and electrolyte replacement for intravenous delivery were the source of the current nosocomial outbreak and physiological state of low immunity (preterm, low birth weight, and mechanical ventilation). The rest of the cases might have been exposed to this organism due to inadequate hand hygiene/improper cleaning and disinfection practices. Timely reporting and implementation of infection control measures can play a significant role in curtailing this outbreak.

Introduction

The *Burkholderia cepacia* complex (BCC) includes 17 Gram-negative rod species that are widely spread in the environment, one of which being *Burkholderia cepacia*. *Burkholderia cepacia* (formerly *Pseudomonas*
B. cepacia is a type of bacteria that can survive and develop in nutrient-poor water and is commonly found in dirt and moist environments. B. Cepacia frequently colonizes the lungs of cystic fibrosis patients and has emerged as a significant opportunistic pathogen in immunocompromised and hospitalized patients. In hospital outbreaks, a single contaminated source, such as disinfectant, intravenous solutions, nebulizer solutions, mouthwash, Normal saline (Dextrose) multi dose antibiotic vials, drinking water, distilled water, flow meters, nasal sprays, and ultrasound gels, is often the source. They pose a risk to people with cystic fibrosis and develop as opportunistic nosocomial microorganisms due to their ability to remain in humid surroundings with hardly sustenance and their intrinsic antiseptic resistance.

Healthcare-associated infections (HAI) are a global problem, but they are more prevalent in developing nations, where they often go unnoticed or unreported. B. cepacia has become a significant human pathogen in the last two decades, producing lethal necrotizing pneumonia and bacteremia, particularly in individuals with cystic fibrosis (CF) or chronic granulomatous illness. The nosocomial spread of this organism is aided by cross-transmission, frequent pulmonary operations, and central venous access, and many nosocomial epidemics in newborns have been documented. The high transmissibility of B. cepacia infection in hospitals, intrinsic resistance to several antibiotics, and link to a poor prognosis underscore the importance of early detection and treatment of B. cepacia infections.

Gram-negative rods, non-fermentative such as Pseudomonas aeruginosa, Acinetobacter baumannii, and Stenotrophomonas maltophilia are common isolates in intensive care units and are key sources of opportunistic infections in hospitalized patients. Multidrug resistance is frequent among these organisms, and treating infections caused by them can be difficult. B. cepacia is a non-fermentative gram-negative bacilli linked to a number of illnesses, including pneumonia, bacteremia, and skin infections.

In India, neonatal septicemia is a leading cause of morbidity and mortality in newborns, with an estimated incidence of 4% in intramural live births. Septicemia in neonates is a widespread bacterial infection that is identified by a positive blood culture in the first four weeks of life, and it is one of Pakistan's top four causes of neonatal mortality. In Pakistan, neonatal septicemia is a leading cause of illness and mortality among newborns.

Bacteremia, urinary tract infections, septic arthritis, peritonitis, and respiratory tract infections are among the ailments characterized by Burkholderia cepacia, which are particularly common in people with Cystic Fibrosis (CF). Due to its intrinsic antibiotic resistance, eradication of B. cepacia is challenging. Changes in the structure of the lipopolysaccharide are among the processes that produce intrinsic resistance. Burkholderia cepacia may be a causative pathogen for nosocomial UTI in pediatric patients with predisposing factors, and appropriate selection of antimicrobial therapy is necessary because of high levels of resistance to empirical therapy, including aminoglycosides. Over 14 years, 14 patients (male-to-female ratio of 1:1) were diagnosed with B. cepacia UTI (urinary Tract Infection). Outside the CF population, infection with B. cepacia complex is poorly understood apart from its presence in occasional
point source outbreaks. Several studies have documented outbreaks among patients with and patients without CF in which patient-to-patient cross-transmission is thought to have occurred(8).

In this study, we described surveillance and successful management of a nosocomial *Burkholderia cepacia* sepsis outbreak in a tertiary care hospital's newborn intensive care unit in Peshawar, Pakistan.

**Methodology**

The NICU of RMI (Rehman Medical Institute) constituted of 60 beds/incubators. Blood specimens for culture were drawn from 50 neonates during outbreak, admitted in NICU with sepsis. If two or more of the following criteria associated with a positive blood culture were met, sepsis was diagnosed: Fever or hypothermia, tachycardia, tachypnea or apnea, and leukocytosis or leukopenia, or a rise in immature forms are all symptoms to look for. Depending on the weight of the neonates, one or two milliliters of blood were obtained using appropriate aseptic protocols. Blood samples were drawn and incubated for a maximum of 7 days at 37°C as part of ongoing surveillance using automated blood culturing systems (BACT/Alert 3D and BACTEC 9050).

Blood culture positives was detected in all samples within 48 hours. These positive samples were gramme stained, revealing Gram negative bacilli that were motile, late oxidase positive, and subcultured on agar using standard procedures, incubated aerobically at 37°C for 48 hours. On Blood agar and MacConkey agar, typical large, circular, low convex, transparent, opaque, glistening colonies were identified after 24 hours, no pigmented at first, later developing yellowish pigmentation colonies. Bacterial identification was also performed using API 20 NE. The isolates' antibiotic resistance pattern was assessed using the Kirby Bauer disc diffusion method using E-strips to determine the lowest inhibitory concentration. Each isolate's turbidity was calibrated with 0.5 McFarland after a single pure colony was inoculated into Muller Hinton Agar and incubated at 37°C for 24 hours. On an inoculation plate, discs impregnated with the proper concentration of antibiotics were applied. The CLSI (Clinical Laboratory Standard Institute) 2021 was used to interpret the results, which were measured to the closest millimeter. Such antibiotics are used to test antibiotic susceptibility, ceftazidime (30µg), meropenem (10µg), levofoxacin (5µg), chloramphenicol (30µg), minocycline (30µg) and co-trimaxazole (25µg).

**Environmental sampling and culture**

To identify the source and path of infection, environmental examinations were carried out. Possible routes for BCC acquisition, such as nutritional sources, incubators, dial flow, flow meter, normal saline water, different medication syrups, bottle feeders, formula milk, and bottle sterilizer, as well as cross transmission through staff nurses, doctors, and ward assistants, are all mentioned in the literature. The following samples were collected as part of the outbreak investigation and cultured on the appropriate Culture Medias.: swabs from sterile incubator, unsterile incubator, dial flow, flow meter, normal saline water, medicine syrups, feeder bottles, and milk and feeder bottle sterilizer. The swabs were plated on Blood agar and MacConkey agar plates and incubated overnight at 36 ± 1°C under aerobic conditions. The next day, the plates were examined for zone of inhibition, and colonies were identified by Gram
staining and biochemical tests using API. *B. cepacia* was isolated from normal saline infusion bottle, which was used for fluid and electrolyte replenishment for intravenous administration. *Staphylococcus* species, *Pseudomonas* species, *Klebsiella* species, and *Bacillus* species were isolated from environmental samples.

## Results

All of 50 patients admitted in NICU were included in this study, and had lately-onset neonatal sepsis, with *B. cepacia*. Ethical approval was obtained from Institutional Ethical review board and written and informed consent was taken from guardians of all the neonates. During an epidemic in the NICU from 30th, April to 21st, June 2021, *B. cepacia* was isolated from over 45 blood samples. Among the isolates which were positive for *Burkholderia cepacia*, 17 (35%) of them were from female neonates while 28 (65%) were male babies. The newborns' average age was 14.88 days. Antibiotic susceptibility testing was performed using Kirby Bauer's disc diffusion method on Muller Hinton agar and MIC (minimum inhibitory concentration) interpreted according to CLSI 2021 guidelines. As *B. cepacia* has intrinsic resistant to polymyxin class (colistin sulphate) of antibiotics, the susceptibility pattern of all isolates were almost similar i.e. showing resistance to tetracycline 100% (minocycline) and cephalosporin 3rd generation 100% (ceftazidime). The isolates were 100% sensitive to fluoroquinolones (levofloxacin), carbapenem 100% (meropenem), chloramphenicol 100% and sulfonamide 100% (co-trimaxazole). Treatment of babies was changed to injection meropenem no fatalities were reported. Figure 1 shows the antibiotic susceptibility of isolate.

## Discussion

An outbreak is considered when an infection is isolated from two or more patients in a defined period and the antibiotic susceptibility pattern is comparable, according to our infection control policy. A neonate with a clinical suspicion of sepsis (fever, tachycardia, tachypnea, leukocytosis, or leukopenia, with or without hypotension) and one or more *BCC*-positive blood culture results was considered an outbreak case. During the study period, 12 newborns were discovered to have *B. cepacia* bacteremia. Seven of the twelve newborns were boys (58.33 percent). The majority of the newborns were premature (83 percent), with a mean gestational age of 34.3 weeks and a range of 30–40 weeks. Ceftazidime (100%) and co-trimoxazole (100%) had the highest susceptibility, followed by chloramphenicol (100%). (91.6 percent). Ciprofloxacin resistance was highest among the isolates(9).

Patients' isolates and environmental samples corresponded to the same biotype and had the same antibiogram, indicating that the isolate was only sensitive to meropenem. According to the Clinical and Laboratory Standards Institute guidelines, the antibiotics effective against BCC include levofloxacin, meropenem, cotrimoxazole, ceftazidime, and minocycline. BCC was isolated from the upper surface of the rubber stopper of sealed multidose amikacin injection vials. It was hypothesized that the needle might have become contaminated while amikacin solution was aspirated from the vials. As per our hospital antibiotic policy, all these babies were started on empirical treatment with intravenous injections of
cefotaxime and amikacin while blood culture results were awaited. This might have been another risk factor in the spread of BCC sepsis, since the organism was resistant to these antimicrobials(2). During September 2016 to February 2017 (six months), a total of 498 blood cultures were sent during febrile episodes. Out of which 60 (12%) came out to be positive for different microorganisms. Out of all positive cultures, Burkholderia cepacia was detected in 29 (48%) patients, which reduced drastically following the change in antibiotic administration practice. All isolates showed sensitivity to piperillin+tazobactum, cefoperazone+sulbactum, fluoroquinolones, cotrimoxazole and carbapenems and resistance to polymyxin B and colistin. With timely intervention by appropriate intravenous antibiotics as per culture sensitivity result and change in antibiotic preparation practice, overall mortality was low 1 (4%) out of 29 culture positive episodes(10).

BCC was found in 35 samples in August 2017. Twenty of the thirty cases were newborns (including 13 neonates). Ceftriaxone (100%), minocycline (95%), chloramphenicol (85%), and cotrimoxazole (84.6%) were the most effective antibiotics, followed by levofloxacin (79.1%), meropenem (71.4%), and ceftazidime (71.4%) (48.3%). At the point of delivery, nineteen children had a low birth weight. The IV catheters were in place in all of the children. Three children (two of whom were neonates) needed to be intubated(11). The median birthweight was 1670 g (range 860–3760) and the median gestational age was 32 weeks (range 27–41) during the first outbreak. There were 32 instances of septicemia, with five patients getting two episodes and two patients having three episodes, respectively. The death rate per septicemic episode was 6.3 percent. Water from an oxygen humidifier in the delivery room, three ventilator water traps, and one humidifier water trap in the neonatal unit were used to isolate the organism. In the second outbreak, six neonates with a median birthweight of 2060 g and a gestational age of 32.5 weeks had septicemia. Two ventilator water traps were used to isolate the bacterium(12).

With a male to female ratio of 2:1, all 12 newborns were out born referrals. The gestation period ranged from 29 to 41 weeks. Four neonates were born with a low birth weight, and two were born with a very low birth weight. Eight patients had early onset sepsis and four had late onset sepsis. Piperacillin/tazobactam, ciprofloxacin, and co-trimoxazole were given to neonates either separately or in combination for 14–21 days, depending on the severity of the accompanying meningitis(13).

Infectious endocarditis caused by B. cepacia is a rare occurrence. Because of respiratory difficulty, a female Indonesian infant was referred to a neonatal critical care unit. On the 23rd day of hospitalisation, an echocardiogram revealed two vegetations on the tricuspid valve, measuring 3.5 mm 2 mm and 2.3 mm 3.4 mm, respectively. Infective endocarditis was diagnosed based on this information. Antibiotic sensitivity tests revealed that the isolate was sensitive to Ceftazidime, Meropenem, and Trimethoprim-sulfamethoxazole, but resistant to Ampicillin, Piperacillin-tazobactam, Amikacin, Gentamicin, Ciprofloxacin, Ampicillin-sulbactam, Cefazolin, and Nitrofurantoin.(14). B. cepacia is also intrinsically resistant to Commercial 0.5% Chlorhexidine solution (chlorhexidine gluconate and chlorhexidine digluconate (CHG) or chlorhexidine acetate) that is a disinfectant and antiseptic used for decontamination. The usage of the 0.5 percent CHG solution in the hospital was halted on January 6, 2015, when it was discovered to be the source of B. cepacia. The entire NICU team was retrained to use
just 10% povidone-iodine as a skin antiseptic. No more B. cepacia was identified from blood once the 0.5 percent CHG product was completely stopped (15).

BCC is becoming more well recognized as a serious human infection in immunocompromised and hospitalized individuals who became infected after coming into contact with contaminated hospital equipment. *B. cepacia* was obtained from the blood cultures of neonates hospitalized to the NICU of a tertiary care hospital in Peshawar in 45 cases of neonatal septicemia. In this study *B. cepacia* was isolated from 30 neonates from 50 suspected neonates which are lately onset and majority are male neonates. *Burkholderia* septicemia in neonates, median birth weight was 1.67 kg and mean, which supports the fact that *Burkholderia* is an opportunistic pathogen causing disease in patients with definite pre-disposing factors. BCC have a unique and challenging antimicrobial profile, which show innately resistant to polymixin. They are showing resistance to minocycline and cephalosporin 3rd generation, and sensitive to fluoroquinolones, carbapenem, chloramphenicol and sulfonamide. When an infection is identified from two or more patients in a specific time range, an outbreak is suspected, according to infection control guidelines. An outbreak was defined in our study as the occurrence of more than two patients with positive BCC culture results at the same time. A neonate with a clinical suspicion of sepsis was defined as an outbreak case. In March 2021, an outbreak was suspected, and an inquiry was launched after frequent cases of bacteremia caused by *B. cepacia* occurred over a three-month period. This prompted the hospital to conduct a full microbiological investigation as well as infection surveillance measures.

**Conclusion**

Intravenous fluids utilized for fluid and electrolyte replacement for intravenous delivery were the source of the current nosocomial outbreak. It is a critical problem that requires immediate intervention, such as single-use IV fluids and intravenous line management. Although *B. cepacia* is not common in hospitals, it is the most common cause of septicemia. For detecting sources of *B. cepacia* contamination and avoiding negative repercussions for immunocompetent and immunocompromised patients, ongoing surveillance and quick examination of atypical disease outbreaks are critical. To control *B. cepacia*-like opportunistic infections, an effective antibiogram is required. The index case in this study may have been exposed to infection as a result of a physiological state of inadequate immunity (preterm, low birth weight, and mechanical ventilation). The remaining instances may have been exposed to this organism as a result of poor hand hygiene and washing and disinfection procedures. The timely reporting of the epidemic and the adoption of infection control measures can help to contain it.

**Declarations**

**Availability of data and materials**

The pathology department at Rehman Medical Institute in Peshawar provided all of the data and resources. If anybody want to get data from this study, they should contact Dr. Maria Khan (Assistant
Professor & Consultant Microbiologist).

**Ethics approval and consent to participate**

The study was approved by the Ethics Committee of Rehman Medical Institute, Peshawar. All procedures were carried out in compliance with the applicable rules and regulations.

**Consent for publication**

Not Applicable.

**Competing interests**

The authors declare that they have no competing/conflict of interests.

**Funding**

Not applicable

**Authors' contributions**

MA collected the clinical and laboratory data.

MK provides Conceptualization and revised the final manuscript.

DA correct and rephrases the manuscript.

FS performed data analysis.

SM and FK drafted the manuscript.

AKA and RN completed Data curation.

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**Consent**

Written and informed consent was taken from guardians of all the neonates.

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**Figures**

![Bar chart showing antibiotic susceptibility of Burkholderia cepacia isolated in neonates admitted to NICU.](image)

**Figure 1**

Antibiotic Susceptibility of patterns of Burkholderia cepacia isolated in neonates admitted to NICU.