Original Research Paper

Medical Microbiology

Dr. Debarati
BanerjeeAssistant Professor, Department Of Microbiology, Calcutta National Medical College.
Medical College.Dr. Rituparna
Haldar*Demonstrator, Department Of Microbiology, Midnapore Medical
Corresponding Author

ABSTRACT Background: The aerobic gram-negative bacillus, Burkholderia cepacia frequently colonizes hospital ward fluids. It is a known important opportunistic pathogen that causes morbidity and mortality due to its intrinsic resistance to most antibiotics used in hospitalized patients, but it poses little risk of infection to healthy individuals. B. cepacia, mainly associated with immunocompromised patients acquire the infection from exposure to contaminated medications, products, and equipments. This observational study has been conducted to identify the source of Burkholderia cepacia infection outbreak that occurred in a tertiary care hospital. Methods: The current study is a prospective observational study done for six months (July 2023 to December 2023). Different respiratory samples collected from Intensive care unit with severe infections were received in our Microbiology department and processed according to standard laboratory guidelines. The study was done with 158 non-repeated samples from clinical specimens like pleural fluid, BAL fluid and sputum from both gender and all age groups of patients attending our hospital. The culture was done by an automated method using BD BACTEC FX 40 and identification and sensitivity pattern for Burkholderia cepacia was done by using Automated Vitek2 compact system. Results: In our study, out of 158 samples, 47 (29%) showed bacterial growth. Out of all positive samples, Burkholderia cepacia was detected in 16 (34%) samples. Conclusion: Our investigation also detected BCC in the water system, which used to manufacture the medical liquid products. An ongoing Burkholderia cepacia outbreak was discovered in a low-resource setting through the use of standardized HAI surveillance. The key components of outbreak management are prompt outbreak identification, source identification, and appropriate outbreak control measures.

KEYWORDS : Burkholderia cepacia, BD BACTEC FX 40, Automated Vitek2 compact system, Respiratory samples, ICU ward, Opportunistic pathogen, Healthcare associated infection.

INTRODUCTION:

Burkholderia cepacia complex, comprising of 20 species, are opportunistic devastating pulmonary pathogens in cystic fibrosis patients and immunocompromised hospitalized patients, has also been reported as an emerging hospital pathogen ^[1,4]. It is an aerobic, catalase negative and nonlactose fermenting gram-negative bacterium. BCC bacteria are found in soil, but they can also live and grow in waterbased environments like lakes, rivers, drinking water, and liquids with trace amounts of nutrients It is frequent colonizer of fluids like intravenous fluids, antiseptic solution etc. used in the hospital ward. Nebulizers, ventilator tubing, and humidifiers have been connected to outbreaks in healthcare facilities^[2]. This bacteria can live for up to a week on dry surfaces, but it can live for many months in water, according to research^[3-5]. B.cepacia is an important nosocomial pathogen that is transmitted to patients when there is re-use of same medical devices without proper sterilization. Due to its inherent resistance to the majority of antibiotics like betalactam antibiotics, aminoglycosides and polymyxin B, B. cepacia infection is a well-known significant opportunistic infection that causes morbidity and mortality in hospitalized patients ^[2,6]. The intrinsic resistance is mediated by various mechanisms which include changed penicillin binding proteins, multiple multi-drug efflux pumps, change in lipopolysaccharide structure and a strong correlation with the emergence of biofilms ^[3]. Due to this natural multidrug resistance property, eradication is challenging.

Burkholderia can infect mainly blood, soft tissues, such as skin, urinary tract, surgical incision sites and catheter insertion sites. The majority of outbreaks have been linked to inappropriate product use, which includes using tainted water in manufacturing processes, diluting antiseptic solutions excessively, using out-of-date products, keeping them open for extended periods of time, and using inappropriate storage conditions^[6]. The transmission related factors are lack of hand-washing, cohorting of cystic fibrosis patients, hand shaking contaminated fomites and contaminated respiratory equipment $^{\scriptscriptstyle [3]}$

MATERIALS AND METHODS

This was a prospective observational study conducted in a tertiary care hospital of eastern India. Total numbers of 158 samples collected from patients with history of respiratory infections and pleural effusion and received in the microbiology department during the period of July 2023 to December 2023 are included in the study. Automated BD BACTEC FX 40 system was used for initial processing of respiratory fluid samples like pleural fluid and BAL fluid. For processing of sputum and respiratory swabs, blood agar, MacConkey's agar and chocolate agar were used. After the initial processing, for growth positive samples further identification and sensitivity were done by VITEK 2 COMPACT System following CLSI guidelines¹⁷¹.

Identification And Antibiotic Susceptibility Test:

- Growth positive samples on BACTEC FX 40 were subcultured onto blood agar media, chocolate agar media, MacConkey agar media and incubated for 18-24 hours at 37°C.
- After 24 hours aerobic incubation on Blood agar plates, large, round, low convex, moist, haemolytic colonies were seen and on MacConkey agar media, non-lactose fermenting colonies were seen.
- Gram negative, motile, oxidase positive bacilli were discovered by gram staining.
- Vitek 2 compact system was used to verify *B. cepacia* isolates. It is composed of four cards such as GN 405+ AST, GN406+ AST, GN628+AST and Yeast ID+ AST. In our experiment we had used GN406+ AST cards. The isolates antibiogram was conducted in compliance with CLSI recommendations. Antibiotic susceptibility tests were performed by vitek2 compact system on Ceftazidime, Meropenem, Levofloxacin, Minocycline, Cotrimoxazole.

VOLUME - 13, ISSUE - 03, MARCH - 2024 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

The data was analyzed and interpreted.

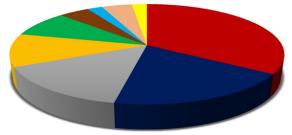
Source of infection:

The outbreak occurred in patients admitted in Intensive Care Unit (ICU). We report the identification of the source as contaminated liquid solutions used in patient care such as nebulizer solutions, ventilator tubing, mouth wash, humidifiers and oxygenation equipment. Water from sink was identified as a primary environmental source and was included in the extensive outbreak control measures. Contaminated solutions used in the patient care activities could cause significant outbreaks. The product was withdrawn from use across the hospital as a corrective measure by the infection control committee.

RESULT:

isolates.

A total of 158 samples (Pleural fluid, BAL fluid, sputum) from ICU ward, were sent to our microbiology department. Out of which 47 (29%) came out to be positive for different microorganisms. Out of all positive cultures, *B. cepacia* was detected in 16 (34%) patients as shown in (Fig1) and table 1.



- Burkholderia cepacia(10.12%) Klebsiella pneumoniae(5.69%)
- Acinetobacter baumannii(4.43%) Staphylococcus aureus(3.16%)
- Escherichia coli(2.53%)

Pseudomonas aeruginosa(1.26%)

Enterococcus faecalis(0.63%)
 Candida albicans (0.63%)

Enterobacter aerogenes(1.26%)

Figl: Distribution of organisms found from respiratory samples according to their frequency of isolation.

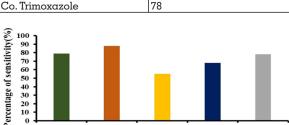
Organism isolated	Number of isolates
Burkholderia cepacia	16
Klebsiella pneumoniae	9
Acinetobacter baumannii	7
Staphylococcus aureus	5
Escherichia coli	4
Pseudomonas aeruginosa	2
Enterococcus faecalis	1
Enterobacter aerogenes	2
Candida albicans	1

Table 1: Organisms found in collected patient's sample

The isolates antibiogram was performed by vitek2 compact system as shown in table2 and Fig2.

Table 2: Result of antibiogram test for Burkholderia cepacia

Antibiotics	Percentage of sensitivity (%)
Ceftazidime	79
Meropenem	88
Levofloxacin	55
Minocycline	68
Co. Trimoxazole	78



Ceftazidime Meropenem Levofloxacin Minocycline Co. Trimoxazole Antibiotics Fig2: Sensitivity Percentage for *Burkholderia* cepacia isolates according to total number of samples.

DISCUSSION:

BCC are opportunistic Gram-negative nosocomial pathogens that can lead to serious outbreaks in clinical settings. The bacteria are highly potential nosocomial pathogens that cause fatal infections in young adult patients because they have an innate ability to survive and multiply in the hospital environment. They grow on moist surfaces like water tanks and others^[2,8].

In our study, we found nine different microorganisms out of 158 samples - Burkholderia cepacia (10.12 %), Staphylococcus aureus (3.16%), Acinetobacter baumannii (4.43 %), Klebsiella pneumoniae (5.69%), Escherichia coli (2.53%), Pseudomonas aeruginosa (1.26%), Enterobacter aerogenes (1.26%), Enterococcus faecalis (0.63%) and Candida albicans (0.63%). Overall B. cepacia is 16 (34%) out of 47(29%) positive samples. However, Baul et al. in 2018 reported that Burkholderia cepacia was detected in 29 (48%) patients out of 60(12%) positive samples in eastern India^[3]. Peterson et al. in 2013 and Abdallah et al. in 2018 looked into a *B. cepacia* pneumonia clonal outbreak in individuals ^(9,10). They determined that the respiratory care items may have been contaminated by the sink^[9]. Similar source of infection have also been identified in our study. In 2017, a nationwide outbreak across Australia of B. cepacia bacteremia was reported by Shaban et al. (2017) The contaminated gel packs in the sachets used in the sterile ultrasound probe covers were found to be the point source of infection. Nine patients developed B. cepacia bacteremia as a result of contaminated analgesic gel used in urological procedures [11]

In addition, our study indicated that B. cepacia, highly sensitive to meropenem (88%) followed by ceftazidime (79%), cotrimoxazole (78%), Minocycline (68%) and levofloxacin (55%). According to a Korean study, the antibiotic susceptibility rates of B. cepacia including meropenem (78.57%), TMP-SMX (71.43%), minocycline (66.67%), ceftazidime (64.29%), and levofloxacin (50%). A study by Chun-Hsing Liao and colleagues found that B. cepacia isolates were sensitive to meropenem (100%), ceftazidime (97.3%), levofloxacin (5.5%) and minocycline (5.5%) in China. All hospital Intensive Care Units (ICUs) are actively monitored for hospital-acquired infections (HAI) by the infection prevention and control (IPC) team. According to other studies, the outbreak was brought to an end by coordinated infection control measures, including enhanced environmental cleaning and disinfection, patient Cohorting, hand hygiene, barrier and contact precautions, visitor restrictions, and outbreak control measures based on good infection control principles^[3]. Similar infection control measures were also followed in our hospital to control such outbreaks.

Patient education programmes emphasise good hygiene habits like washing hands thoroughly, wearing a mask when coughing and not sharing objects like toothbrushes and handkerchiefs. Regular environmental monitoring for contamination involves taking water samples from drains and showers, as well as cultures from fitness, physiotherapy and pulse oximeter equipment.

CONCLUSION:

B. cepacia is an opportunistic pathogen that was first identified in patients with lung involvement in cystic fibrosis or chronic granulomatous disease. It typically results from product or device contamination. Infection control is particularly problematic because of its ubiquitous growth pattern in different water sources of hospitals and inherent resistance to many antibiotics, antiseptic solutions, and disinfectants ^[2-3]. There are few reports of BCC outbreaks involving Intensive care Unit patients, but a lot of them come from oncology departments, and haemodialysis units. Any clinical ward experiencing Burkholderia sepsis should be taken seriously because it could be a sign of inadequate barrier nursing care and a violation of sepsis guidelines. Through persistent monitoring and proactive oversight, this atypical pandemic could be controlled, safeguarding the immunocompromised patients in ICU ward who are at risk.

A prompt investigation into the outbreak was made possible in this study by the proactive hospital infection control team and focused information regarding the potential source of contamination. To identify additional cases, it is essential to promptly notify public health authorities of such events and disseminate information related to the outbreak. Monitoring B. cepacia infections is essential because they can change their epidemiology and lead to increased resistance. Using effective antibiotics like trimethoprim-sulfamethoxazole, meropenem, and ceftazidime, along with close observation of infections, can lead to a favourable prognosis.

Acknowledgement:

The author would like to acknowledge Dr. Ajay Kumar Ray, Principal, Calcutta National Medical College & Hospital and Dr. Prof. Baishali Chakraborty, Head of the Department, Department of Microbiology, CNMC&H, for their consistent support for the necessary approval for this study.

Conflict of interest: The authors declare that there is no known conflict of interest associated with this publication.

Abbreviations:

BCC: Burkholderia cepacia complex BAL: Bronchoalveolar lavage ICU: Intensive Care Unit HAI: Hospital-Acquired Infections IPC: Infection Prevention and Control CDC: Centers for Disease Control

REFERENCES:

- Häfliger E, Atkinson A, Marschall J. Systematic review of healthcareassociated Burkholderia cepacia complex outbreaks: presentation, causes and outbreak control. Infection prevention in practice. 2020 Sep 1;2(3):100082.
- Bilgin H, Altınkanat Gelmez G, Bayrakdar F, Sayın E, Gül F, Pazar N, Çulha G, Süzük Yıldız S, Cinel I, Korten V. An outbreak investigation of Burkholderia cepacia infections related with contaminated chlorhexidine mouthwash solution in a tertiary care center in Turkey. Antimicrobial Resistance & Infection Control. 2021 Dec; 10(1):1-6.
- Baul SN, De R, Mandal PK, Roy S, Dolai TK, Chakrabarti P. Outbreak of Burkholderia cepacia infection: a systematic study in a hematolooncology unit of a tertiary care hospital from eastern India. Mediterranean journal of hematology and infectious diseases. 2018;10(1).
- Coenye T, Vandamme P, Govan JR, LiPuma JJ. Taxonomy and identification of the Burkholderia cepacia complex. Journal of clinical microbiology. 2001 Oct 1;39(10):3427-36.
- Vandamme P. Dawyndt P. Classification and identification of the Burkholderia cepacia complex: past, present and future. Systematic and applied microbiology. 2011 Apr 1;34(2):87-95.
- Wanger A, Chavez V, Huang R, Wahed A, Dasgupta A, Actor JK. Microbiology and molecular diagnosis in pathology: a comprehensive review for board preparation, certification and clinical practice.
 Batool A, Yaqoob A, Anwar Z, Joshi LT, Batool R, Lone D, Saleem Z, Ahmed Q,
- Batool A, Yaqoob A, Anwar Z, Joshi LT, Batool R, Lone D, Saleem Z, Ahmed Q, Bin Jardan YA, Bourhia M, Qamar MU. Outbreak investigation of NDMproducing Burkholderia cepacia causing neonatal sepsis in Pakistan. Future Microbiology. 2023 Nov;18(16):1159-69.
- Microbiology. 2023 Nov;18(16):1159-69.
 Okomo U, Senghore M, Darboe S, Bojang E, Zaman SM, Hossain MJ, Nwakanma D, Le Doare K, Holt KE, Hos NJ, Lawn JE. Investigation of sequential outbreaks of Burkholderia cepacia and multidrug-resistant extended spectrum -lactamase producing Klebsiella species in a West African tertiary hospital neonatal unit: a retrospective genomic analysis. The Lancet Microbe. 2020 Jul 1;1(3):e119-29.
- Peterson AE, Chitnis AS, Xiang N, Scaletta JM, Geist R, Schwartz J, DeMent J, Lawlor E, LiPuma JJ, O'Connell H, Noble-Wang J. Clonally related Burkholderia contaminants among ventilated patients without cystic fibrosis. American journal of infection control. 2013 Dec 1;41(12):1298-300.
- Abdallah M, Abdallah HA, Memish ZA. Burkholderia cepacia complex outbreaks among non-cystic fibrosis patients in the intensive care units: a review of adult and pediatric literature. Infez Med. 2018 Dec 1;26(4):299-307.
- 11. Shaban RZ, Maloney S, Gerrard J, Collignon P, Macbeth D, Cruickshank M, Hume A, Jennison AV, Graham RM, Bergh H, Wilson HL. Outbreak of health care-associated Burkholderia cenocepacia bacteremia and infection attributed to contaminated sterile gel used for central line insertion under ultrasound guidance and other procedures. American journal of infection control. 2017 Sep 1;45(9):954-8.