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Original Research Article

Clinical Isolates Identification of Burkholderia Species and Evaluate Their Antibiogram at a Tertiary Care Hospital

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Abstract

Background: The genus Burkholderia, which was previously believed to be a soil saprophyte, is now gaining attention as a human disease. Pathogenic species include B. pseudomallei, B. cepacia, B. mallei, B. gladioli, and B. thailandensis. Ongoing studies on Bukholderia have led to the isolation of more recent subspecies from human samples. The purpose of this study is to assess the antibiogram of the clinical isolates and determine the prevalence of Burkholderia spp.

Methods: Demographic data and a variety of patient clinical samples were analyzed. All specimens were processed using recognized microbiological techniques.

Results: Of the 8230 culture-positive samples, 1902 (23.11%) included Non-Fermenting Gram Negative Bacilli (NFGNB). It was discovered that 60 (3.2%) of these NFGNB were Burkholderia spp. Furthermore, it was found that 24 (40%) and 36 (60%) were B. pseudomallei and B. cepacia complex, respectively. Of the patients, 76.7% were over 40, and 80% were men. Diabetes mellitus was the primary risk factor (60%), and fever was the most common manifestation (53.3%). Imipenam had the lowest sensitivity, while Minocycline and Cotrimoxazole had the highest, according to antibiotic sensitivity testing.

Conclusion: This study provides baseline data on the present Burkholderia infection situation at our hospital. An ongoing investigation will be useful in identifying several cases because this organism is extremely underreported.

Keywords: NFGNB, Burkholderia infection, Risk factors, Prevalence, Antibiogram.

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Introduction

Non-fermenting gram negative bacilli (NFGNB) are a broad category of opportunistic pathogens that neither oxidatively digest nor use carbohydrates as a source of energy.[1] In hospital settings, they are now opportunistic pathogens that cause meningitis, pneumonia, septicemia, urinary tract infections (UTI), surgical site infections (SSI), and other health care-associated infections (HAI).[2] Pseudomonas aeruginosa is the most common NFGNB, followed by Acinetobacter calcoaceticus baumanni complex, Pseudomonas fluorescence, Pseudomonas stutzeri, Stenotrophomonas maltophilia, Pseudomonas putida, and Burkholderia cepacia complex.[3] In 1942, Walter Burkholder characterized the Burkholderia species, which is a member of the _ proteobacteria, as a phytopathogenic microbe that affects onions and carnations. It is a member of the Burkholderiaceae family, which includes a variety of species, including "friends and foes."[4,5] The biotechnological and agricultural sectors frequently employ some of the beneficial Burkholderia species for bioremediation and biocontrol. In endemic areas like Southeast Asia and Northern Australia, these gram-negative saprophytes inhabit the soil, water reservoirs, and rhizosphere.[6] Although there are currently over 99 species in the genus, Burkholderia cepacia complex (BCC), B. psuedomallei, and B. mallei are the three most significant diseases to people and animals. Burkholderia mallei causes glanders disease, B. pseudomallei causes melioidosis, and BCC is a nosocomial pathogen that can infect people with cystic fibrosis.[7] In addition to the three Burkholderia species mentioned above, Burkholderia thailandensis, B. fungorum, and B. gladioli have also been isolated from clinical samples [8,9]. We selected to investigate the

incidence of these group of organisms in our hospital since they are either severely underreported as causal agents or incorrectly reported as Pseudomonas species.

Materials and Methods

From March 2025 to February 2026, this study was carried out at the Department of Microbiology at the Mahatma Gandhi Memorial Medical College and Hospital, Jamshedpur, Jharkhand.

A wide range of patient clinical samples were examined. All of the specimens were inoculated into Sheep Blood agar (5%) and Mac Conkey agar after being microscopically examined using Gram staining. For at least five days, the plates were incubated aerobically at 370C. The shape, growth, and biochemical reactions of the colonies were used to identify the organisms. Based on the oxidase test, resistance to Polymyxin B, Colistin, and Gentamicin, as well as other pertinent conventional biochemical reactions such as oxidative utilization of glucose, lactose, mannitol, and maltose, liquefaction of gelatin, reduction of nitrate, and hydrolysis of arginine, the isolates of NFGNBs were further identified as Burkholderia species. Additional identification.BD phoenix was used to confirm the Burkholderia species.

Clinical presentations, underlying risk factors, and other demographic information were examined in case records of individuals infected with Burkholderia spp.

Antibiotic sensitivity testing was performed on the clinical isolates of Burkholderia spp. in accordance with the CLSI recommendations 2024.[10] Sensitivity testing was carried out using Kirby Bauer's disk diffusion method with the appropriate antibiotics after the isolates were put onto Mueller Hinton's agar.

Result

1902 of the 8230 culture-positive samples that were examined in our investigation were found to be Non Fermenting Gram Negative Bacilli. 60 isolates of these NFGNBs were biochemically determined to be Burkholderia spp. Twenty-four (40%) of the sixty Burkholderia isolates were further identified as B. pseudomallei, while the remaining thirty-six (60%) were classified as B. cepacia.

The table below shows the distribution of B. pseudomallei and cepacia isolates in the different clinical samples other than urine. Because there were no Burkholderia isolates in the urine samples examined at this time, the results did not include them. Patients from whom Burkholderia spp. were isolated were further classified as either nonbacteremic (localized) or bacteremic (blood culture positive with one or no discernible site of infection). Eight (57.1%) of the 24 B. pseudomallei isolates were recovered from blood alone, four (28.6%) from blood and pus sample, and two (14.3%) from blood and knee aspirate. Of the 14 cases of B.cepacia, 38.9% developed bacteremia, of which 12 (85.7%) were isolated from blood alone and 2 (14.3%) from blood plus bronchoscopic washing.

Table 1: Distribution of Burkholderia spp. among various samples

Sample	B. pseudomallei	B. cepacia
Blood	8	12
Pus	6	10
Pus + Blood	4	-
Blood + Body fluids	2	2
Body fluids (stomach aspirate)	2	-
Sputum	2	6
Bronchoscopic washing and ET secretions	-	6

48 (80%) of the patients in this study were men, indicating a male predominance. 46 (76.7%) of the Burkholderia-infected individuals were older than 80, according to the age distribution analysis.

Table 2: Gender Distribution of Burkholderia spp. among infection

Gender	B. pseudomallei n(%)	B. cepacian(%)
Male	22(92.0%)	26(72%)
Female	2(8.0%)	10(28%)

Table 3: Age wise Distribution of Burkholderia spp. among infection

Age (in years)	B. pseudomallei n(%)	B. cepacian(%)
0-20	2(8.0%)	4(16.0%)
21-40	4(16.0%)	4(16.0%)
>40	18(76.0%)	28(68.0%)

Diabetes Mellitus Type II (DM) was identified as the primary risk factor for Burkholderia infection in 36 patients (60%), and smokers and drinkers accounted for 12 cases (23.3%). Eight patients (13.3%) had no underlying risk factors. Risk variables overlapped in the majority of infected patients.

Table 4: Predisposing factors in this Burkholderia case

Risk Factors	B. pseudomallei n(%)	B. cepacian(%)
Diabetes Mellitus	16(66.7%)	20(55.6%)
Alcoholic/smoking	6(25%)	6(16.7%)
COPD/TB	2(8.3%)	10(27.8%)
CKD	2(8.3%)	4(11.1%)
Trauma	-	2(5.6%)
Preterm	2(8.3%)	-
Others	-	4(13%)
No risk factors	2	6

Patients with Burkholderia spp. infection had varied clinical presentations of which fever (32 cases- 53.3%) was the major presentation. Respiratory tract infections were seen in 26 (43.3%) cases and their presenting symptoms included cough, breathlessness, respiratory distress, pneumonia and 2 case of hydropneumothorax. Abscess and soft tissue infections was seen in 16 (27.6%) of the patients. There was overlapping of presentations in many of the cases.

Table 5: Clinical presentation of patients with Burkholderia isolates

Presentation	B. pseudomallei n(%)	B. cepacian(%)
Fever	12(50.0%)	20(55.6%)
Respiratory Symptoms	8(33.3%)	18(50.0%)
Abscess and soft tissue infections	10(41.6%)	6(16.7%)

46 (76.7%) of the 60 Burkholderia spp. isolates came from patients who had shown up. 34 (56.7%) of the infected individuals worked in a variety of occupations, including mining and farming. Of these 34 patients, 22 had B. cepacia infection (61.1%) and 12 had melioidosis (50%).

Kirby Baeur's disc diffusion method was used to determine the antibiotic sensitivity of B. pseudomallei in accordance with CLSI 2019 criteria. Ceftazidime (CAZ), Imipenem (IPM), Meropenem (MRP), Minocycline (MI), Levofloxacin (LE), Amoxiclav (AMC), and Cotrimoxazole (COT) were among the antibiotic discs utilized for testing. Doxycycline should be utilized for B. pseudomallei sensitivity testing, per CLSI 2019 guidelines. Doxycycline's sensitivity could not be evaluated since it was absent from the panel of antibiotics. Every strain of B. pseudomallei exhibited 100% sensitivity to minocycline and cotrimoxazole, 91.6% sensitivity to levofloxacin, 83.3% sensitivity to amoxiclav and ceftazidime, 75% sensitivity to meropenem, and 58.3% sensitivity to imipenem. According to CLSI 2019 recommendations, an antibiogram for B.cepacia was performed in Vitek II compact (bioMerieux).

Ceftazidime (CAZ), Imipenem (IPM), Meropenem (MRP), Minocycline (MI), Levofloxacin (LE), ticarcillin-clavulanate, and Cotrimoxazole (COT) were among the antibiotic discs selected. The 18 strains were all 100% sensitive to cotrimoxazole and minocycline, 94.4% sensitive to levofloxacin, 77.8% sensitive to ceftazidime and meropenam, and 38.8% sensitive to imipenam. One strain had intermediate susceptibility to ceftazidime, while three strains (16.7%) shown resistance.

Table 6: Antibiogram of Burkholderia pseudomlleiisolates

Discs	Sensitive	Intermediate	Resistant
Ceftazidime (Caz)	20	4	-
Imipenem (Ipm)	14	2	8
Meropenem (Mrp)	18	-	6
Cotrimoxazole (Cot)	24	-	-
Amoxiclav (Amc)	20	-	4
Levofloxacin (Le)	22	2	-
Minocycline (Mi)	24	-	-

Table 7: Antibiogram of Burkholderia cepaciaisolates

Discs	Sensitive	Intermediate	Resistant
Ceftazidime (Caz)	28	2	6
Imipenem (Ipm)	14	2	22
Meropenem (Mrp)	28	2	6
Cotrimoxazole (Cot)	36	-	-
Ticarcillin+clavulanate (Ti)	28	-	8
Levofloxacin (Le)	34	2	-
Minocycline (Mi)	36	-	-

Discussion

Burkholderia is a member of rRNA group II and is a non-fermenting gram negative bacilli (NFGNB). Its tolerance to the polymyxin group of antibiotics (polymyxin B 300 g and colistin 10 g) sets it apart from pseudomonads. 60 isolates of Burkholderia spp. (3.1%) were found throughout our investigation, including B. pseudomallei and B. cepacia. This was comparable to a study conducted by Hu Yan Jian, who found that 2.9% of Burkholderia species were isolated.[11]

The prevalence of B. pseudomallei and B. cepacia was examined by sample. Of the 24 B. pseudomallei isolates, 10 patients (41.7%) displayed signs of localized melioidosis, and 14 patients (58.3%) developed bacteremia. This was comparable to research by Suputtamongkol [12], which found that the incidence of bacteremic melioidosis was 58%, while Cheng and Currie's investigation found that the incidence of localized melioidosis was 44.8%.[13] Of the 36 B. cepacia isolates, 14 patients (38.9%) experienced bacteremia. In a research by Reik and LiPuma, 15.5% of B. cepacia isolates had bacteremia.[14] 61.1% of the B.cepacia isolates in our analysis also displayed localized infection.

French Indo-China first identified B. pseudomallei's saprophytic characteristics in 1955. Additionally, Australian sample investigations have revealed that bacterial populations rise between 60 and 90 cm below the surface.

In their investigation, Currie and Jacups attributed the higher isolation of Burkholeria spp. during the monsoon to the bacteria's migration from the deeper soil layers to the surface due to the rising water table.[16] In a similar vein, Ramsay et al. discovered that B. cepacia cases were more common during periods of severe rainfall.[17]

Cheng and Currie's research on the connection between occupation and melioidosis revealed that those who were exposed to surface water and soil at work or during their leisure time had a higher incidence of the disease, especially when farmland and rice fields flooded.[13] Of the patients with melioidosis in our study, 50% had an occupational relationship; most of them worked in construction, agriculture, or fishing. Studies by Vidyalaksmi et al. [15], Beena et al. [18], and Currie et al. [13] similarly revealed a greater incidence of exposure among agricultural workers and outdoor maintenance personnel. There are no validated studies that link employment to contracting B. cepacia. However, 61.1% of our patients worked in construction and agriculture. Since B. cepacia is a soil saprophyte, the clinical strains of cepacia would have been obtained from the natural environment, according to Ludovic et al. [19]. In our study, males had a greater percentage of Burkholderia spp. infection (80%), with 45.8% of males having B. pseudomallei infection and 54.2% having cepacia infection.

In her article, Vidyalakshmi et al. [15] justified this by pointing out that men are more likely to engage in outdoor activities. In contrast to our findings, which showed that only 28% of female patients had B.cepacia infection, Rahbar et al.'s study [20] on the disease found that there were more female patients (58.3%) than male patients (41.7%).

Our study's age-wise statistics revealed a median age of 52.2, with the oldest participant being 79 years old and the youngest being a 3-day-old child. In line with previous research, the most prevalent age group displayed was over 40 (76.7%). In line with Vidyalaksmi et al.'s findings (75.8%), 75% of melioidosis cases involved people over 40.[15] 77.8% of patients with B.cepacia infections were older than 40.

Since Burkholderia spp. are known to cause illness in both healthy and immunocompromised people, underlying risk factors for infection have been investigated.[21] Diabetes mellitus is a significant underlying risk factor among individuals infected with this bacterium, according to research conducted globally and in our study. The rising incidence of this lifestyle illness may be a factor in the rise in bacterial infections. According to our research, 80% of patients with Burkholderia spp. infections had one or more risk factors, with diabetes mellitus (DM) accounting for 60% of these cases. DM was discovered to be the primary risk factor when additional risk variables for melioidosis patients were examined. Diabetes mellitus was identified as the primary risk factor in studies from melioidosis-endemic areas, with regional variations in its prevalence. According to

Vidyalakshmi et al.'s study [15], 75.8% of the participants had diabetes, which seems to be the greatest percentage ever recorded. A Thai study linked deficient polymorphonuclear leukocytes (PMNLS) to the higher frequency of melioidosis in diabetic individuals. When compared to PMNLs in patients without diabetes, this leads to decreased migration in response to interleukin-8, poor phagocytosis of the organism, and an inability to postpone apoptosis. [22]

The next significant risk factor identified was TB and chronic obstructive pulmonary disease. This risk factor was present in 8.3% of melioidosis patients in our analysis, compared to 4% in Saravu et al.'s study.[23] Among our patients with Burkholderia infection, chronic kidney disease (CKD) was also identified as a risk factor. We found that 8.3% of our melioidosis patients had renal illness, which is similar with the 8% of instances in Mukhopadhyay et al. study.

There were no documented cases of B. cepacia infection in our investigation, in contrast to other studies where cystic fibrosis was identified as the primary risk factor. In our analysis, DM was identified as the primary risk factor for B. cepacia infection, with COPD and TB coming in second and third, respectively, at 27.8%. According to a study by Matthaiou et al. [24], people with COPD may have pulmonary lesions that serve as habitats for chronic colonizers. This suggests that B.cepacia is a chronic colonizer that may induce lung disease in patients who do not have cystic fibrosis. In our study, 11.1% of B.cepacia patients had CKD, whereas Bressler [25] had 20%.CKD is a risk factor for cepacia patients.

When the various clinical manifestations of Burkholderia spp. infection were examined, fever was discovered to be the most common presenting symptom (53.3%). Abscesses and soft tissue infections (27.8%) and respiratory symptoms (43.3%) were other presentations. Studies by Gopalakrishnan et al. (65%) and Saravu (80%) also found that 50% of melioidosis patients had fever.[23, 26] Abscesses and soft tissue infections were the next most common manifestation among patients with melioidosis (41.6%), including one case of perigastric abscess and one of epidural abscess. According to a research by Mukhopadhyay et al. [23], 32% of patients with melioidosis exhibited soft tissue and skin involvement.

According to Cheng's melioidosis page, abscesses can spread hematogenously and become a cause of systemic infection. The next most common symptom in 33.3% (4 cases) of melioidosis patients was respiratory illness. Only two of the four patients with respiratory infections had radiographic evidence, while the other two had positive blood cultures or sputum. In a Thai series, it was previously reported that 40% of melioidosis patients with normal chest radiographs had positive sputum cultures without radiologic abnormalities. From their sputum, pseudomallei was isolated. These patients weren't really sick. This is probably not an accidental finding of colonizing B. pseudomallei, but rather respiratory tract melioidosis at the milder end of the spectrum.[27]

Numerous illnesses, including as pneumonia, bacteremia, and infections of the skin and soft tissues, are linked to B. cepacia. Fever was the most common symptom among the B.cepacia-infected individuals in our study (55.6%), followed by respiratory symptoms (50%) and soft tissue infections (16.7%). Additionally, compared to patients infected with B. pseudomallei, our investigation revealed that patients infected with B. cepacia experienced more respiratory symptoms.

The higher degree of colonization by B. cepaciain the upper respiratory tract accounts for the increased respiratory symptom which could be the reason for this finding.

Burkholderia species' antibiogram was examined. The organism was shown to be most sensitive to Cotrimoxazole and Minocycline (100%) and least sensitive to Imipenem (46.7%). Meropenem (76.7%), ceftazidime and ticarcillin clavulanate (80%), and levofloxacin (93.3%) all shown sensitivity. Ceftazidime is the recommended medication for Burkholderia infections, according to the majority of research. Three isolates (10%) in our investigation exhibited ceftazidime resistance. Doxycycline's sensitivity pattern against B. pseudomallei could not be evaluated due to its unavailability in the panel of antibiotics.

Ten deaths (16.7%) were observed in our investigation, and all of the patients had B. cepacia infections. Patients with melioidosis did not report any deaths or relapses. Eight (17.4%) of the ten patients who passed away were older than 40 and had risk factors such as COPD, CKD, cancer, and neurological disorders. Since all of these individuals had underlying risk factors that could have contributed to their deaths, there was insufficient clinical evidence to conclude that the fatalities were caused by B. cepacia infections. To identify Burkholderia infection as the main cause of death among persons infected with this organism, more research with a larger sample size over a longer time period will be needed.

Conclusion

Our knowledge of the organism's basic biology and pathology has advanced, but our comprehension of its ecology and epidemiology is still lacking. Blood, soft tissue, and respiratory tract infections are caused by Burkholderia spp. Since it is

impossible to diagnose infections caused by this bacteria clinically, the majority of cases globally likely go undiagnosed because they affect people with limited access to diagnostic facilities.

It becomes necessary to identify these germs through laboratory diagnosis. Many times, the aetiological agent is reported as NFGNB or simply as Pseudomonas species because there are no sophisticated diagnostic resources to confirm it. All NFGNB cultivated in clinical specimens should be speciated to aid in diagnosis.

These organisms are still susceptible to many of the prescribed antibiotics, according to a number of investigations, including ours. Their infections respond well to medications, but melioidosis necessitates a prolonged course of treatment lasting up to 12 weeks. For the majority of patients, a combination of a high index of suspicion, culture confirmation, and timely and adequate therapy with approved medications will yield an excellent outcome.

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